International Journal of Pharmaceutics, 62 (1990) 193–205
Elsevier

IJP 02100

Prodrugs as drug delivery systems. 107. Synthesis and chemical and enzymatic hydrolysis kinetics of various mono- and diester prodrugs of *N*-acetylcysteine

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(Received 24 December 1989)
(Accepted 27 January 1990)

Key words: Prodrug; *N*-Acetylcysteine; Thioester; Ester; Stability; Enzymatic hydrolysis

Summary

The systemic bioavailability of *N*-acetylcysteine (NAC) following oral administration is very low due to extensive first-pass metabolism by deacetylation, primarily in the gut. In an attempt to depress this metabolism and hence increase the systemic bioavailability various esters of NAC were synthesized and assessed as prodrug forms for the parent drug. The esters studied included alkyl esters, glycolamide esters and an acyloxymethyl ester formed at the carboxylic acid part of NAC as well as the *S*-benzoyl ester and various carboxylic acid esters thereof. An *S*-benzoyl carbamate ester was also studied. The pH-rate profiles for the hydrolysis of the derivatives were obtained at 37°C and interpreted in terms of hydrogen ion-, hydroxide ion- and water-catalyzed reactions. The hydrolysis of the oxy- and thioesters was enzymatically catalyzed by human and rat plasma as well as by rat liver and intestinal homogenate. The chemical and enzymic hydrolysis characteristics of the esters suggest that esterification of NAC at the carboxylic acid function or at the thiol group, or both, may be a potentially useful means to obtain prodrug forms of the drug for protecting it against intestinal deacetylation.

Introduction

N-Acetyl-L-cysteine (NAC) (**I**) is widely used as a mucolytic agent (Sheffner, 1963; Boman et al., 1983; Millar et al., 1985), as a chemoprotective adjunct in cancer therapy (Yarbro, 1983) and as an antidote in paracetamol intoxication (Prescott et al., 1977). Whereas the mechanism of action of NAC with regard to protection against liver damage caused by paracetamol overdosage is believed to involve its function as a precursor for glutathione synthesis (Lauterburg et al., 1983; Corcoran and Wong, 1986), less is known about the mechanisms underlying the effects of NAC observed in the treatment of chronic bronchitis. Thus, it is not known whether the activity is due to NAC itself or to any of its metabolites such as L-cysteine and glutathione.

The systemic bioavailability of NAC following oral administration to humans is only about 5–10% and shows large interindividual variations (Borgström et al., 1986; Cotgreave and Moldéus, 1987; Olsson et al., 1988; Burgunder et al., 1989; Caro et al., 1989). The low bioavailability is not due to incomplete absorption (Bonamoni and Gazzaniga,

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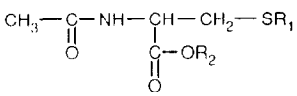
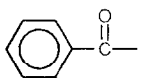
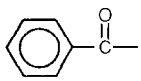
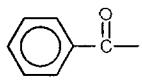
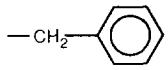
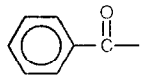
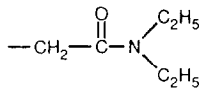
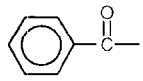
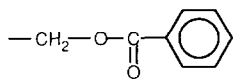
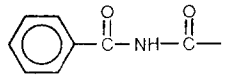
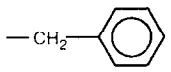
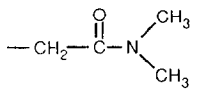
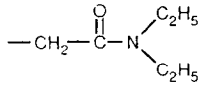
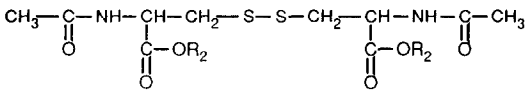
1980), but rather to extensive first-pass metabolism in the gut and/or the liver. Thus, Cotgreave et al. (1987) have shown that NAC is rapidly deacetylated to L-cysteine by the small intestine in rats and more recently, Sjödin et al. (1989) have demonstrated that NAC is effectively deacetylated by rat, mouse and human liver, lung and intestinal homogenates and that the deacetylation in the intestinal mucosa and possibly in the intestinal lumen is the major factor determining the low oral bioavailability of NAC. The deacetylation which is probably effected by an α-*N*-acyl-L-amino acid



	R_1	R_2
I	H	H
II		H
III		$-C_2H_5$
IV		
V		
VI		
VII		H
VIII	H	$-C_2H_5$
IX	H	
X	H	
XI	H	



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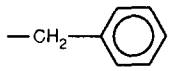
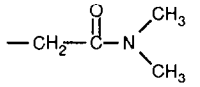
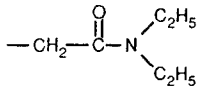
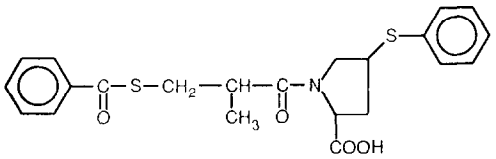
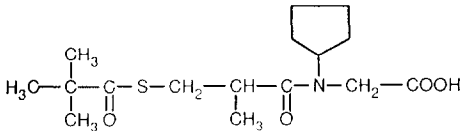
XIII	R_2 =	
XIV	R_2 =	
XV	R_2 =	



XVI



XVII

hydrolase was further found to be a rather specific process as *N*-acetyl-D-cysteine and the disulphide of NAC were resistant to hydrolysis (Sjödin et al., 1989). The clinical relevance of the deacetylation and hence the low systemic bioavailability of NAC is uncertain and may be dependent on the pharmacological use of drug. Thus, this metabolic step may be a prerequisite for the protective effect of the compound in paracetamol intoxication through its function as a cysteine and glutathione precursor (Burgunder et al., 1989) whereas the mucolytic effects, on the other hand, might be enhanced through decreased first-pass metabolism and improved delivery of the intact drug to lung tissues.

A means to study this question may be testing of prodrug forms of NAC which are capable of protecting the drug against deacetylation in the liver and especially in the intestine, yet being cleaved to the parent drug after entrance into the systemic circulation or following pulmonary uptake. NAC contains a carboxylic acid and a thiol group and both of these groups are readily amenable to bioreversible derivatization by, e.g. esterification (Bundgaard, 1985). Since the deacetylation of NAC appears to be a rather specific process (Sjödin et al., 1989) it may be imagined that transient modification of the molecule at the thiol and/or the carboxylic acid function could block or depress this metabolic degradation.

The present paper is the first part of such investigations and describes the synthesis, chemical stability and enzyme-mediated conversion of various derivatives of NAC (**II–XV**). Since only limited information is available on prodrug forms of the thiol function (Bundgaard, 1985), the results of this study with NAC prodrugs formed at its thiol group may also have a broader interest in terms of prodrug modification of SH-containing agents.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu system consisting of an LC-6A pump, an SPD-6A variable-wavelength UV detector and 20 μl loop injection valve. In some cases, a Kontron instrument consisting of an LC Pump T-414, a Uvikon 740 UV detector operated at a fixed wavelength (240 or 215 nm) and a 20 μl loop injection valve was used. Deactivated Supelcosil LC-8-DB reversed-phase columns (33 × 4.6 mm) (3 μm particles) equipped with Supelguard columns (Supelco Inc., U.S.A.) were used. The pH measurements were made at the temperature of study using a Radiometer Type PHM 83 instrument. Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of N-acetylcysteine derivatives (II–XV)

S-Benzoyl-N-acetylcysteine (II) **II** was prepared by reacting NAC with benzoyl chloride as described by Portelli et al. (1976); m.p. 199–200 °C (from ethanol), reported m.p. 200–201 °C (Portelli et al., 1976).

S-Benzoyl-N-acetylcysteine ethyl ester (III) Ethyl iodide (0.41 ml, 5 mmol) was added to a solution of *S*-benzoyl-*N*-acetylcysteine (1.07 g, 4 mmol) and triethylamine (0.72 ml, 5 mmol) in 5 ml of *N*,*N*-dimethylformamide. The mixture was stirred at room temperature for 20 h and poured into 50 ml of water. The precipitate formed upon standing at 4 °C for 4 h was filtered off, washed with water and recrystallized from ethanol-water to give 0.9 g of compound **III**.

S-Benzoyl-N-acetylcysteine benzyl ester (IV) **IV** was prepared from compound **II** and benzyl bromide in a similar way to that described for compound **III**.

S-Benzoyl-N-acetylcysteine N,N-diethylglycolamide ester (V) 2-Chloro-*N*,*N*-diethylacetamide (0.82 ml, 6 mmol) was added to a mixture of compound **II** (1.34 g, 5 mmol), triethylamine (0.90 ml, 6.5 mmol) and sodium iodide (90 mg, 0.6 mmol) in 5 ml of *N*,*N*-dimethylformamide. The mixture was stirred at room temperature for 24 h, poured into water (60 ml) and extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with a 2% aqueous sodium bicarbonate solution and water, dried over anhydrous sodium sulphate and evaporated in vacuo. The residue

TABLE 1

Melting points and analytical data of various ester derivatives of N-acetylcysteine

Compound	M.p. (°C)	Formula	Analysis (%)		
				Calculated	Found
III	128–129	$C_{14}H_{16}NO_4S$	C	57.13	57.25
			H	5.48	5.50
			N	4.76	4.74
IV	90– 92	$C_{19}H_{19}NO_4S$	C	63.85	63.65
			H	5.36	5.38
			N	3.92	3.90
V	101–102	$C_{18}H_{24}N_2O_5S$	C	56.82	56.84
			H	6.36	6.34
			N	7.36	7.31
			S	8.43	8.30
VI	119–120	$C_{20}H_{19}NO_6S$	C	59.84	59.80
			H	4.77	4.83
			N	3.49	3.45
VII	177–178	$C_{13}H_{14}N_2O_5S$	C	50.32	50.32
			H	4.55	4.65
			N	9.03	8.96
XII	124–125 [a]	$C_{14}H_{24}N_2O_6S_2$	C	44.20	44.31
			H	6.36	6.40
			N	7.36	7.31
XIII	140–141	$C_{24}H_{28}N_2O_6S_2$	C	57.12	57.02
			H	5.59	5.70
			N	5.55	5.68
XIV	116–117	$C_{18}H_{30}N_4O_8S_2 \cdot 2.5\ H_2O$	C	40.06	40.07
			H	6.53	6.62
			N	10.38	10.43
XV	80– 81	$C_{22}H_{38}N_4O_8S_2 \cdot 1\ H_2O$	C	46.47	46.37
			H	7.09	7.12
			N	9.85	9.75

[a] Reported m.p. 124.5 °C (Hollander and Du Vigneaud, 1931).

obtained was recrystallized from ethyl acetate-ether-petroleum ether to give 1.1 g of the title compound.

S-Benzoyl-N-acetylcysteine benzoyloxymethyl ester (VI) A mixture of compound **II** (0.80 g, 3 mmol), triethylamine (0.55 ml, 4 mmol), sodium iodide (45 mg, 0.3 mmol) and benzoyloxymethyl chloride (0.47 ml, 3.3 mmol) (prepared as described by Binderup and Hansen (1984)) in *N,N*-dimethylformamide (3 ml) was stirred at room temperature for 24 h and poured into water (50 ml). The precipitate formed was filtered off, washed with water and recrystallized from ethanol-water to give 0.91 g of compound **VI**.

S-Benzoyl carbamate ester of N-acetylcysteine (VII) A solution of benzoyl isocyanate (0.74 g, 5 mmol) (from Aldrich) in acetonitrile (5 ml) was added with stirring to a hot (50–60 °C) solution of *N*-acetylcysteine (0.82 g, 5 mmol) in acetonitrile (10 ml). The mixture was stirred for 2 h at room temperature and the precipitate formed filtered off. Upon recrystallization from ethanol compound **VII** was obtained in a yield of 60%.

Esters XII–XV of N,N-diacetyl-L-cystine **XII–XV** were prepared by esterification of *N,N*-diacetyl-L-cystine with the appropriate alkyl halogenide as described above for compound **V**. *N,N*-Diacetyl-L-cystine was prepared as described by Marshall et al. (1957). Compounds **XII, XIV** and **XV** were recrystallized from water and compound **XIII** from ethanol-water.

Esters VII–XI of N-acetylcysteine These were prepared by reduction of the corresponding esters of *N,N*-diacetylcystine (**XII–XV**) with dithio-

threitol (Cleland, 1964; Field and Giles, 1971). A typical procedure was as follows: Compound **XV** (1.71 g, 3 mmol) was dissolved in a mixture of ethanol (3 ml) and water (17 ml). The pH of the solution was adjusted to 8 by addition of 2 M sodium hydroxide. Dithiothreitol (0.92 g, 6 mmol) was added and the resulting solution stirred at room temperature for 30 min, the pH being maintained at 8–8.5. Hydrochloric acid (4 M) was added to bring the pH of the reaction solution to 2.5 and the solution was evaporated in vacuo. The oily residue obtained resisted crystallization. HPLC analysis of the residue showed a content of less than 1% of the starting disulfide.

Physical and analytical data for the derivatives **III–VII** and **XII–XV** are given in Table 1. The NMR spectra of all the compounds **II–XV** were consistent with their structures.

Analysis of N-acetylcysteine and its derivatives

N-Acetylcysteine and the derivatives **II–XV** were quantitated by reversed-phase HPLC procedures capable of separating the compounds from the products of degradation. A deactivated Supelcosil column was eluted with mobile phase systems consisting of 5–40% v/v of acetonitrile or 5–50% v/v of methanol in 0.1% phosphoric acid, the concentration of acetonitrile or methanol being adjusted for each compound to give an appropriate retention time (2–8 min). The flow rate was 1.0–2.0 ml min^{-1} and the column effluent was monitored at 240 nm or, for compounds **I** and **VIII–XV**, at 215 nm. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

Kinetic measurements

Hydrolysis in aqueous solution The hydrolysis of the *N*-acetylcysteine derivatives was studied in aqueous buffer solutions at 37.0 ± 0.2° C. The buffers used were hydrochloric acid, acetate, phosphate, borate, carbonate and sodium hydroxide 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. In the case of the compounds **VIII–XI** dithiothreitol (10^{-4} M) was added to the solutions as a protective reagent to maintain the thiols in the reduced state.

The reactions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10.0 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 10^{-4} M. The solutions were kept in a water bath at 37° C and at appropriate intervals samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual ester against time.

For slowly proceeding reactions (compound **II** at pH 1–7.4) the rate constants were obtained by measuring the initial rates of benzoic acid formation. The initial concentration of the ester **II** was 5×10^{-4} M. The formation of benzoic acid was followed up to 1–3% of the initial concentration. Pseudo-first-order rate constants for the hydrolysis were obtained by dividing the slopes of linear plots of benzoic acid formed vs time with the initial (and almost constant) ester concentration.

Stability in biological media The hydrolysis of the derivatives was studied at 37° C in human and rat plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4 as well as in 10% rat liver homogenate. The latter was prepared as previously described (Buur and Bundgaard, 1984). The initial concentration of the compounds was about 10^{-4} M. The mixtures were kept in a water bath at 37° C and at appropriate intervals samples of 500 μl were withdrawn and deproteinized by adding 500 μl of a 2% solution of zinc sulphate in methanol-water (1 : 1 v/v). For compounds **VIII–XI**, however, deproteination was performed by adding 250 μl of a 10^{-2} M solution of dithiothreitol in 5% perchloric acid to the 250 μl samples (cf. Johansson and Westerlund (1987)). After mixing and centrifugation at 13000 rpm for 3 min, 20 μl of the clear supernatant was chromatographed as described above. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

The hydrolysis of the esters **II** and **V** was also studied in 20% rat intestinal homogenate. Samples

were analyzed as described for the plasma studies. The rat intestines (jejunum and ileum) were washed with phosphate-buffered saline (pH 7.4) and homogenized in this medium at 4 °C.

Results and Discussion

Hydrolysis of the NAC derivatives II–XI in buffer solutions

The kinetics of hydrolytic breakdown of the NAC esters **II–VI** and **VIII–XI** as well as of the *S*-benzoyl carbamate derivative **VII** was studied in aqueous buffer solution of pH 7.4 at 37 °C. For some representative compounds (**II, V, VII** and **XI**) the kinetics of hydrolysis was studied over the pH range 1–11. At constant pH and temperature the disappearance of the derivatives followed strict first-order kinetics over several half-lives as determined by HPLC. In the case of the esters **VIII–XI** dithiothreitol was added to the reaction solutions at a concentration of 10^{-4} M in order to inhibit oxidation of the thiol group to disulphide. At the buffer concentration used (0.02 M) no significant general acid-base catalysis was observed.

The influence of pH on the overall rates of degradation of the derivatives **II–VI** is shown in Fig. 1 where the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. As seen from Fig. 1 the pH-rate profiles for compound **II** and **V** are U-shaped, indicating the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction according to the following rate expression:

$$k_{obs} = k_H a_H + k_0 + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = \text{pH} - 13.62 \quad (2)$$

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) catalyzed hydrolysis were determined from the straight line portions of the pH-rate profiles at low and high pH values, respectively, whereas values of the first-order rate constant for spontaneous hydrolysis (k_0) were obtained on the basis of Eqn 1. The values of the rate constants derived are listed in Table 2. In Fig. 1 the solid curves or lines drawn were constructed from these constants and Eqn 1.

TABLE 2

Rate data for the overall hydrolysis of various esters of N-acetylcysteine in aqueous solution at 37 °C and $\mu = 0.5$

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	K_{OH} (M^{-1} min^{-1})
II	3.4×10^{-5}	2.5×10^{-6}	20
III	–	–	250
IV	–	–	815
V	4.7×10^{-3}	3.1×10^{-5}	1.8×10^{3}
VI	–	1.8×10^{-3}	1.6×10^{4}
VIII	–	–	141 (k'_{OH})
IX	–	–	222 (k'_{OH})
X	–	–	119 (k'_{OH})
XI	1.0×10^{-3}	2.8×10^{-5}	2.4×10^{3} (k_{OH}) 34 (k'_{OH})

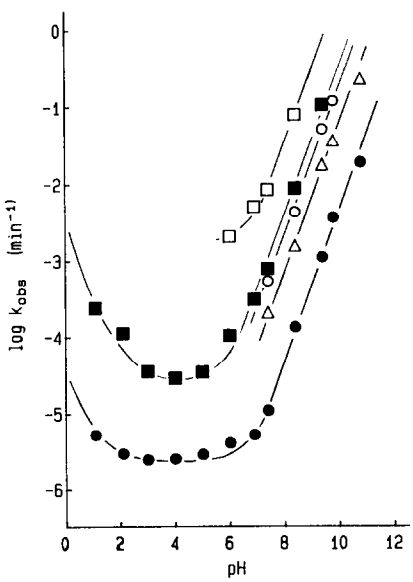


Fig. 1. The pH-rate profiles for the hydrolysis of the *N*-acetylcysteine esters **II** (●), **III** (△), **IV** (○), **V** (■) and **VI** (□) in aqueous solution at 37 °C ($\mu = 0.5$).

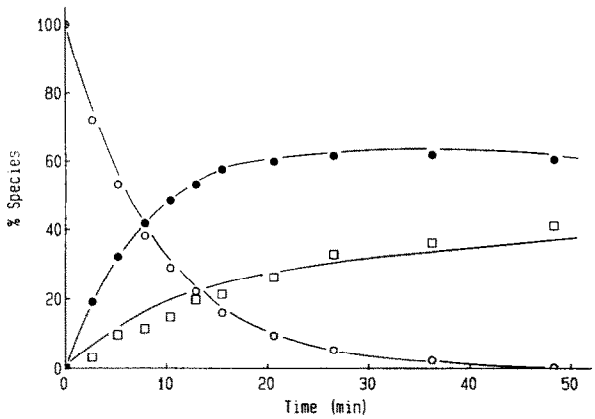
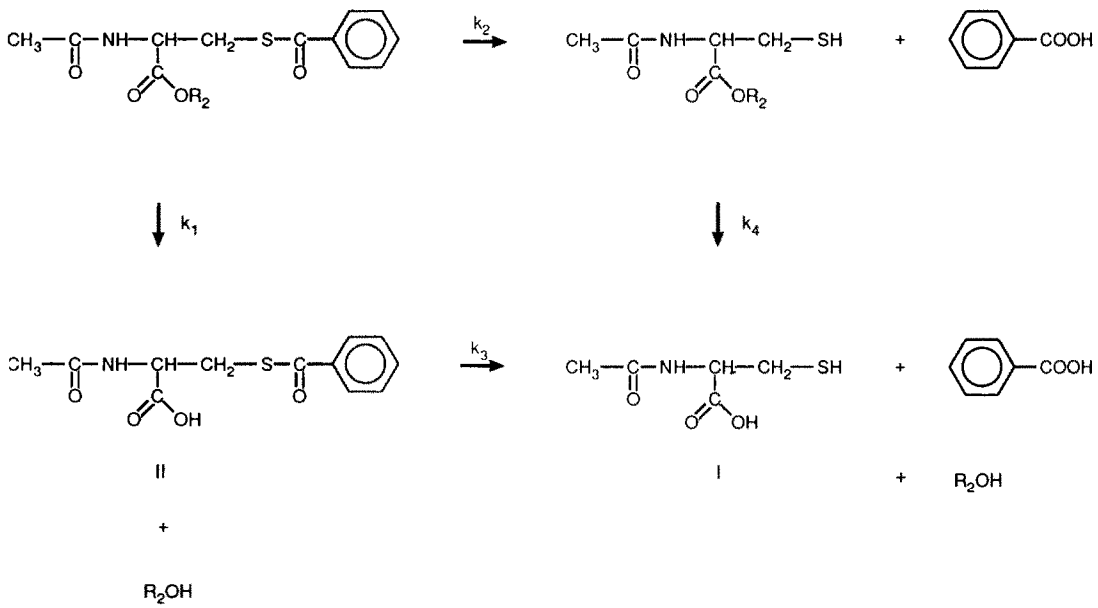


Fig. 2. Time courses for compound **IV** (○), compound **II** (●) and benzoic acid (□) in the degradation of compound **IV** at pH 9.80 and 37 °C. The concentrations at various times, expressed as per cent of the initial concentration of **IV**, were determined by HPLC.

Analysis of the reaction solutions of the thioester **II** by HPLC revealed a quantitative formation of benzoic acid. Thus, the rate constants for this compound solely refer to hydrolysis of the thioester moiety. The lack of a curvature in the pH-rate profile for compound **II** at pH values around the pK_a value for carboxyl groups apparently indicates that the hydrolytic reactivity of the thioester bond is largely independent of the form (ionic or undissociated) of the carboxylate group.

The diesters **III–VI** may, on the other hand, be susceptible to undergo simultaneous hydrolysis at both the thioester and oxyester moieties as depicted in Scheme 1, where k_1–k_4 are pseudo-first-order rate constants for the depicted reactions. Using HPLC procedures enabling quantitation of the diesters **III–VI** and their hydrolysis products benzoic acid and *S*-benzoyl NAC (**II**) the course of degradation of the diesters was examined at pH 8.4–9.8. In all cases the disappearance of the diesters was found to be accompanied by the formation of *S*-benzoyl NAC (**II**) and benzoic acid. Following its formation compound **II** degraded slowly to benzoic acid and NAC, both identified on the basis of their HPLC retention behaviour in comparison with those of authentic samples. The time-course of the various species during degradation of the diester **IV** is shown in Fig. 2. At any reaction time the sum of diester **IV** and the hydrolysis products **II** and benzoic acid corresponds to $100 \pm 5\%$. Analysis of such data showed that the k_1-reaction involving cleavage of the oxyester moiety is the dominating degradation route for the diesters **III–V** in alkaline solution,



Scheme 1.

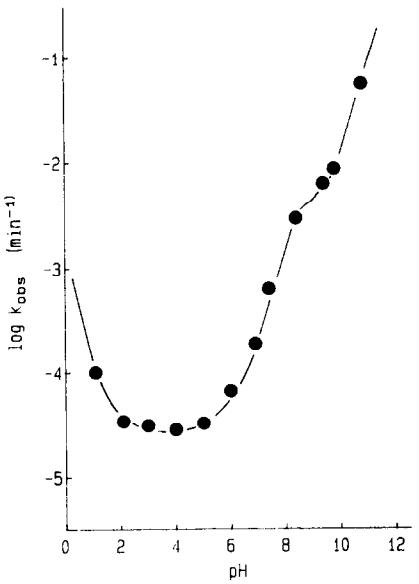


Fig. 3. The pH-rate profile for the hydrolysis of the *N*-acetylcysteine ester **XI** in aqueous solution at 37 °C ($\mu = 0.5$).

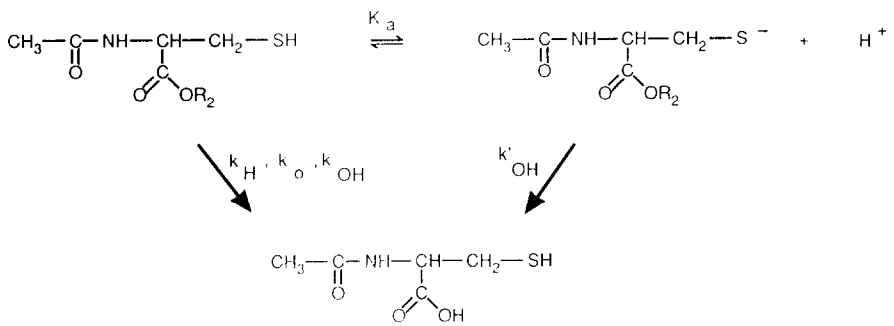
accounting for 55% (**III**), 65% (**IV**), 85% (**V**) and 92% (**VI**) of the total degradation. Since k_3 is much smaller than the rate constant for the overall loss of diester ($k_{obs} = k_1 + k_2$), the rate constants k_1 and k_2 and hence the percentage contributions of these routes were simply determined on the basis of the relative amounts of ester **II** and benzoic acid formed during the initial 1–2 half-lives of diester hydrolysis. Since benzoic acid also arises from hydrolysis of the oxyester function in diester **VI** the k_1/k_{obs} ratio for this compound was determined from the amount of ester **II** formed. The relative stability of the diesters **III**–**VI** in alkaline solution is in accordance with the different stabilities of similar benzoic esters, the difference being accountable for in terms of the different polar effects within the alcohol moieties (Nielsen and Bundgaard, 1987).

The pH-rate profile for the hydrolysis of the monoester of NAC (**XI**) at 37 °C is shown in Fig. 3. The shape of the pH-rate profile indicates that the hydrolysis can be described according to the following rate expression:

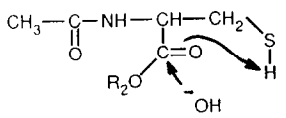
$$k_{obs} = k_H a_H + k_0 + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (3)$$

where K_a is the apparent ionization constant of the thiol group in the ester and k_{OH} and k'_{OH} are the second-order rate constants for the apparent hydroxide ion-catalyzed hydrolysis of the undissociated and ionized species, respectively (Scheme 2). The values of the rate constants derived from Fig. 3 are listed in Table 2. The kinetically derived pK_a value for the SH-group in compound **XI** (8.35) is in the expected range. The pK_a value of the SH-group in *N*-acetylcysteine methyl ester is 8.7 at 23 °C (Snyder, 1984).

Comparison of the k_{OH} and k'_{OH} values for **XI** shows that the ester with an undissociated thiol group is about 35 times more reactive toward alkaline hydrolysis than the ionized form. Such a rate difference can be attributed to electrostatic repulsion by the thiolate anion of the hydroxide ion attack on the ester carbonyl or by some kind of intramolecular facilitation or catalysis of the



Scheme 2.



Scheme 3.

undissociated SH-group. The latter may eventually consist of a general acid-specific base-catalyzed hydrolysis as depicted in Scheme 3.

N-Acylated carbamate esters of alcohols and phenols have recently been studied as potential prodrug derivatives (Bundgaard et al., 1988); since no data are available on the chemical reactivity of such compounds derived from thiols the *S*-benzoyl carbamate ester of NAC (**VII**) was included in this study. In aqueous solution compound **VII** was found to be quantitatively hydrolyzed to NAC and benzamide (Fig. 4). The pH-rate profile for the hydrolysis is shown in Fig. 5 and can be accounted for by the following rate expression:

$$k_{obs} = k_H a_H + k_0 + k_0' \frac{K_a}{a_H + K_a} \qquad (4)$$

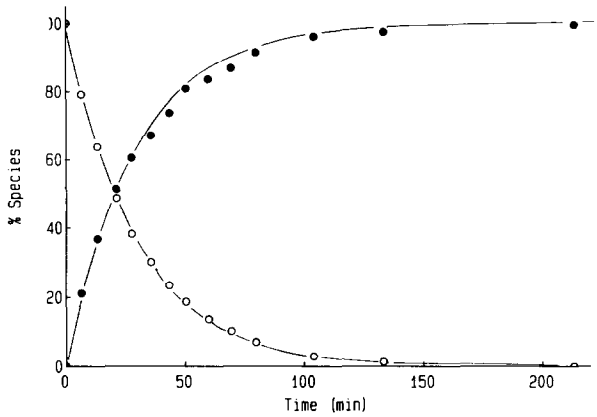
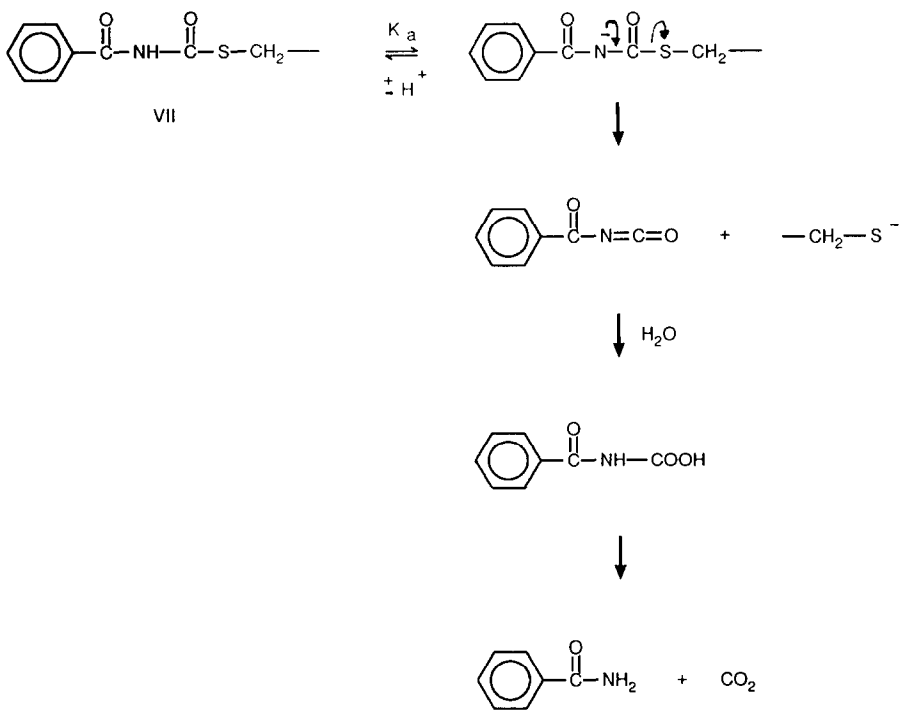


Fig. 4. Plots showing the time-course of degradation of compound **VII** (○) and the simultaneous formation of benzamide (●) at pH 7.40 and 37 °C.

where k_0 represents water-catalyzed hydrolysis of undissociated **VII**, k_0' spontaneous decomposition of the anionic form of **VII** and K_a is the apparent ionization constant of the carbamate NH-group in the compound. From the pH-rate profile shown in



Scheme 4.

Fig. 5, the following rate and ionization constants were obtained (at 37 °C):

$k_H = 6.6 \times 10^{-4}\ M^{-1}\ min^{-1}$

$k_0 = 1.0 \times 10^{-5}\ min^{-1}$

$k_0' = 0.63\ min^{-1}$

$pK_a = 8.6$

Analogous with the behaviour of *N*-acyl carbamate esters of phenols (Bergon and Calmon, 1976; Moravcova and Vecera, 1977) an E1cB elimination mechanism involving an unstable isocyanate intermediate (Scheme 4) may most likely be involved for compound **VII** in neutral and alkaline solutions. The pK_a of the thiol group in NAC (9.51 at 23 °C and $\mu = 1.0$ (Snyder, 1984)) is in the same range as that of phenols and since the E1cB mechanism with the conjugate base of the carbamate being spontaneously split in the rate-determining step predominates for the phenol esters and the $B_{AC}2$ mechanism for *N*-acyl carbamate esters of alcohols with $pK_a >$ approx. 11 (Bergon and Calmon, 1976), the former mechanism is strongly favoured. Additional support for this conclusion is provided by the very similar values of the rate constant k_0' for compound **VII** (0.63 min^{-1}) and phenyl benzoylcarbamate (1.3 min^{-1} at 37 °C, $pK_a = 8.9$) (Bundgaard et al., 1988).

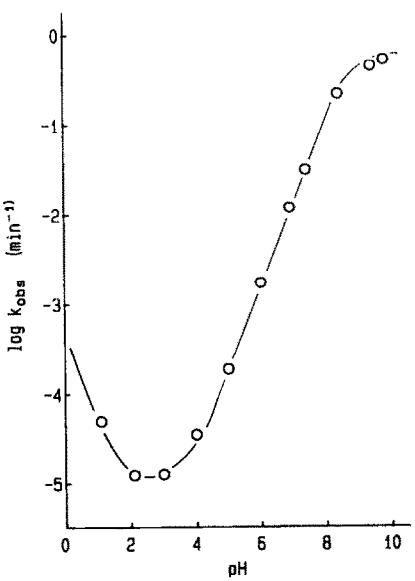


Fig. 5. The pH-rate profile for the decomposition of the *S*-benzoyl carbamate ester derivative **VII** in aqueous solution at 37 °C ($\mu = 0.5$).

TABLE 3

Half-lives for the hydrolysis of various N-acetylcysteine derivatives in aqueous buffer solution and biological media (pH 7.4) at 37 °C

Compound	Half-lives			
	pH 7.4 buffer	80% human plasma	80% rat plasma	10% rat liver homogenate
II	1.1×10^3 h	>100 h	18 h	25 min
III	55 h	2.2 h		0.3 min
IV	22 h	24 min		< 5 s
V	15 h	23 min	< 5 s	0.2 min
VI	1.4 h	5 min		< 5 s
VII	22 min			
VIII		65 min	3.4 min	
IX		48 min	< 5 s	
X		34 min	1.4 min	
XI	18 h	30 min	1.4 min	

Enzymatic hydrolysis of the NAC derivatives

The susceptibility of the various mono- and diesters of NAC to undergo a potential enzymatic hydrolysis was studied at 37 °C in 0.01 M phosphate buffer solutions (pH 7.40) containing 80% human or rat plasma and in the supernatant fraction (diluted to 10% with the pH 7.4 buffer) of rat liver homogenate. The observed half-lives for the hydrolysis of the derivatives in these biological media are listed in Table 3 along with the half-lives of degradation in pure buffer solutions of pH 7.40. As appears from the rate data both plasma and rat liver enzymes markedly accelerate the rate of hydrolysis. The degradation course of the diesters **III**–**VI** was studied in a similar way as that described above for the runs performed in aqueous buffer solutions and found to be as depicted in Scheme 1. However, the relative importance of the k_1 and k_2 reactions was different from that in alkaline aqueous solutions. In 80% human plasma solutions compounds **III** and **IV** were predominantly hydrolyzed at the thioester group as ben-

zoic acid and the *S*-benzoyl ester **II** were found to be formed in yields of about 80 and 20%, respectively. For the diesters **V** and **VI**, on the other hand, the *S*-benzoyl ester **II** was the predominant degradation product, being formed to an extent of about 88% from **V** and > 95% from **VI**. In rat plasma, the diester **V** underwent hydrolysis quantitatively and very rapidly ($t_{1/2} < 5$ s) to the *S*-benzoyl ester **II**. Thus, the carboxylic acid ester moieties in **V** and **VI** appear to be hydrolyzed enzymatically faster than the ethyl and benzyl ester moieties in **III** and **IV**. The greater reactivity of the *N*,*N*-diethylglycolamide ester function toward enzymatic hydrolysis as compared with the ethyl ester is in accordance with the behaviour of similar esters of benzoic acid and other carboxylic acids (Bundgaard and Nielsen, 1988; Nielsen and Bundgaard, 1988; Steffansen et al., 1989). The facile enzymatic hydrolysis of the *O*-benzoyloxymethyl ester **VI** is also to be expected in view of the behaviour of *O*-acyloxymethyl esters of various other carboxylic acids (Bundgaard, 1985; Bundgaard et al., 1989).

The facile decomposition of the diesters **III**–**VI** in 10% rat liver homogenate (Table 3) was found to be due to hydrolysis of the carboxylic acid ester moiety in the compounds since the *S*-benzoyl ester **II** was formed in quantitative amounts. Following its formation this derivative is hydrolyzed to yield benzoic acid and hence NAC, the half-life being 25 min (Fig. 6).

Compounds **II** and **V** were also studied in 20% rat intestinal homogenate. Whereas compound **II** showed no measurable degradation following incubation at 37 °C for 3 h, the diester **V** was quickly degraded, the half-life being 3.1 min. The reaction taking place is hydrolysis of the glycolamide ester group, since the *S*-benzoyl ester **II** was shown to be formed in quantitative amounts.

These data on the liver and intestinal homogenate-catalyzed degradation of the NAC derivatives suggest that, by blocking the thiol and/or carboxylic acid group of NAC, the *N*-deacetylation of the drug effected by intestinal and liver enzymes can be at least transiently hindered or depressed.

Considering the enzymatic hydrolysis of the thioester grouping in compounds **II**–**VI** it is interesting to observe the dependence of the reactivity of this group on the form of the carboxylic acid moiety. Thus, whereas the thioester in compound **II** is highly resistant to enzymatic hydrolysis in human plasma, it is quite facilely hydrolyzed in compounds **III** and **IV**. As described above, the k_2 reaction involving cleavage of the thioester is the predominant degradation pathway for these diesters in human plasma. Thus, it can be calculated that the half-life of hydrolysis of the thioester group in **III** and **IV** in human plasma is 2.8 and 0.5 h, respectively. The high resistance of compound **II** toward enzymatic hydrolysis by plasma may be due to its negative charge at physiological pH, since it is well-known that carboxylic acid esters with an ionized carboxylate group generally are poor substrates for plasma hydrolytic enzymes (Nielsen and Bundgaard, 1987).

Thiol esters have previously been used as prodrug forms for thiols. Thus, the angiotensin-converting enzyme inhibitors zofenopril (**XVI**) and pivopril (**XVII**) are *S*-benzoyl and *S*-pivaloyl esters, respectively, functioning as prodrugs. The parent drugs contain a carboxylic acid group in addition to the thiol group and in agreement with the data reported here for compound **II** both zofenopril and pivopril are only slowly hydrolyzed to their parent drugs in plasma and in vivo (Suh et al., 1985; Jemal et al., 1988).

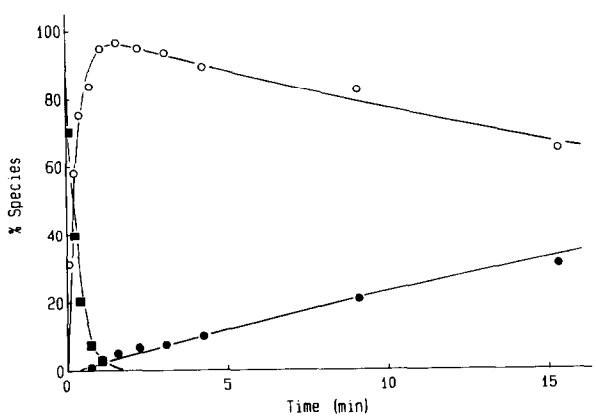


Fig. 6. Plot showing the time course of degradation of compound **V** (■) and the formation of compound **II** (○) and benzoic acid (●) during incubation of compund **V** in 10% rat liver homogenate at 37 °C.

Acknowledgements

This work was supported by Pharma Biotec Research Centre and the Lundbeck Foundation.

References

Bergon, M. and Calmon, J.-P., Hydrolyse alcaline de *N*-acétylcarbamates. Changement de mécanisme lié à la nature du groupe ester. *Bull. Soc. Chim. Fr.*, (1976) 797–802.

Binderup, E. and Hansen, E.T., Chlorosulfates as reagents in the synthesis of carboxylic acid esters under phase-transfer conditions. *Synth. Comm.*, 14 (1984) 857–864.

Boman, G., Bäcker, U., Larsson, S., Melander, B. and Wåhlinder, L., Oral acetylcysteine reduces exacerbation rates in chronic bronchitis: report of a trial organized by the Swedish Society for Pulmonary Diseases. *Eur. J. Resp. Dis.*, 64 (1983) 405–415.

Bonanomi, L. and Gazzaniga, A., Toxicological, pharmacokinetic and metabolic studies on acetylcysteine. *Eur. J. Resp. Dis.*, 61 (Suppl. 111) (1980) 45–51.

Borgström, L., Kågedal, B. and Paulsen, O., Pharmacokinetics of *N*-acetylcysteine in man. *Eur. J. Clin. Pharmacol.*, 31 (1986) 217–222.

Bundgaard, H., Design of prodrugs: bioreversible derivatives of various functional groups and chemical entities. In H. Bundgaard (Ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 1–92.

Bundgaard, H., Buur, A. and Lee, V.H.L., Timolol prodrugs: preparation and hydrolysis kinetics of *N*-benzoyl carbamate esters of timolol and related compounds. *Acta Pharm. Suec.*, 25 (1988) 293–306.

Bundgaard, H., Mørk, N. and Hoelgaard, A., Enhanced delivery of nalidixic acid through human skin via acyloxymethyl ester prodrugs. *Int. J. Pharm.*, 55 (1989) 91–97.

Bundgaard, H. and Nielsen, N.M., Glycolamide esters as a novel biolabile prodrug type for non-steroidal anti-inflammatory carboxylic acid drugs. *Int. J. Pharm.*, 43 (1988) 101–110.

Burgunder, J.M., Varriale, A. and Lauterburg, B.H., Effect of *N*-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. *Eur. J. Clin. Pharmacol.*, 36 (1989) 127–131.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. I. Hydrolysis kinetics and physicochemical properties of various *N*-acyl derivatives of 5-fluorouracil. *Int. J. Pharm.*, 21 (1984) 349–364.

Caro, L.D., Ghizzi, A., Costa, R., Longo, A., Ventresca, G.P. and Lodola, E., Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. *Arzneim. Forsch.*, 39 (1989) 382–386.

Cleland, W.W., Dithiothreitol, a new protective reagent for SH groups. *Biochemistry*, 3 (1964) 480–482.

Corcoran, G.B. and Wong, B.K., Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by *N*-acetyl-L-cysteine in vivo: Studies with *N*-acetyl-D-cysteine in mice. *J. Pharmacol. Exp. Ther.*, 253 (1986) 54–61.

Cotgreave, I.A., Berggren, M.A., Jones, T.W., Dawson, J. and Moldéus, P., Gastrointestinal metabolism of *N*-acetylcysteine in the rat, including an assay for sulfite in biological systems. *Biopharm. Drug Disp.*, 8 (1987) 377–386.

Cotgreave, I.A. and Moldéus, P., Methodologies for the analysis of reduced and oxidized *N*-acetylcysteine in biological systems. *Biopharm. Drug Disp.*, 8 (1987) 365–375.

Field, L. and Giles, P.M., Biologically oriented organic sulfur chemistry. VI. Uses of *o*-carboxyphenyl *o*-carboxybenzenethiosulfonate with thiols. *J. Org. Chem.*, 36 (1971) 309–313.

Harned, H.S. and Hamer, W.J., The ionization constant of water in potassium chloride solutions from electromotive forces of cells without liquid junction. *J. Am. Chem. Soc.*, 55 (1933) 2194–2206.

Hollander, L. and Du Vigneaud, V., The resolution of inactive cystine and isolation of pure dextrorotatory cystine. *J. Biol. Chem.*, 94 (1931) 243–252.

Jemal, M., Ivashkiv, E., Teitz, D. and Cohen, A.I., Simultaneous determination of the prodrug zofenopril and its active drug in plasma by capillary gas chromatography-mass-selective detection. *J. Chromatogr.*, 428 (1988) 81–92.

Johansson, M. and Westerlund, D., Determination of *N*-acetylcysteine, intact and oxidized, in plasma by column liquid chromatography and post-column derivatization. *J. Chromatogr.*, 385 (1987) 343–356.

Lauterburg, B.H., Corcoran, G.B. and Mitchell, J.R., Mechanism of action of *N*-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. *J. Clin. Invest.*, 71 (1983) 980–991.

Marshall, R., Winitz, M., Birnbaum, S.M. and Greenstein, J.P., Preparation and properties of the isomeric forms of cystine and *S*-benzylpenicillamine. *J. Am. Chem. Soc.*, 79 (1957) 4538–4544.

Millar, A., Pavia, D., Agnew, J.E., Lopez-Vidriero, M.T., Lauque, D. and Clarke, W., Effect of oral *N*-acetylcysteine on mucus clearance. *Br. J. Dis. Chest*, 79 (1985) 262–266.

Moravcova, J. and Vecera, M., Kinetics and mechanism of hydrolysis of aryl *N*-trichloroacetylcarbamates. *Collect. Czech. Chem. Commun.*, 42 (1977) 3048–3056.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: a reference system for designing prodrug esters of carboxylic acid agents. *Int. J. Pharm.*, 39 (1987) 75–85.

Nielsen, N.M. and Bundgaard, H., Glycolamide esters as biolabile prodrugs of carboxylic acid agents: synthesis, stability, bioconversion, and physicochemical properties. *J. Pharm. Sci.*, 77 (1988) 285–298.

Olsson, B., Johansson, M., Gabrielsson, J. and Bolme, P., Pharmacokinetics and bioavailability of reduced and oxidized *N*-acetylcysteine. *Eur. J. Clin. Pharmacol.*, 34 (1988) 77–82.

Portelli, M., Renzi, G., Cervato, G. and Frigeni, V., Synthesis of *N*-acetylcysteine compounds. *Il Farmaco, Ed. Sci.*, 31 (1976) 767–775.

Prescott, L.F., Park, J., Ballantyne, A., Adriaenssens, P. and Proudfoot, A.T., Treatment of paracetamol (acetamino-

phen) poisoning with *N*-acetylcysteine. *Lancet*, ii (1977) 432–434.

Sheffner, A.L., The reduction in vitro in viscosity of mucoprotein solutions by a new mucolytic agent, *N*-acetyl-L-cysteine. *Ann. N.Y. Acad. Sci.*, 106 (1963) 298–310.

Sjödin, K., Nilsson, E., Hallberg, A. and Tunek, A., Metabolism of *N*-acetyl-L-cysteine. Some structural requirements for the deacetylation and consequences for the oral bioavailability. *Biochem. Pharmacol.* (1989).

Snyder, G.H., Free energy relationships for thiol-disulfide interchange reactions between charged molecules in 50% methanol. *J. Biol. Chem.*, 259 (1984) 7468–7472.

Steffansen, B., Mørk, N. and Bundgaard, H., Prodrugs as drug delivery systems. 95. Stability and enzymatic hydrolysis of glycolate, glycolamide and alkyl esters of tyrosine. *Acta Pharm. Nord.*, 1 (1989) 47–56.

Suh, J.T., Skiles, J.W., Williams, B.E., Youssefyeh, R.D., Jones, H., Loev, B., Neiss, E.S., Schwab, A., Mann, W.S., Khandwala, A., Wolf, P.S. and Weinryb, I., Angiotensin-converting enzyme inhibitors. New orally active antihypertensive (mercaptoalkanoyl)- and [(acylthio)alkanoyl]glycine derivatives. *J. Med. Chem.*, 28 (1985) 57–66.

Yarbro, J.W., *N*-Acetylcysteine (NAC): A significant chemoprotective adjunct. Proceedings of a symposium. *Semin. Oncol.*, 10 (Suppl. 1) (1983) 1–92.