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Prodrug forms for the sulfonamide group. III. Chemical and enzymatic hydrolysis of various *N*-sulfonyl imidates — a novel prodrug form for a sulfonamide group or an ester function

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Summary

The kinetics and mechanism of degradation of a series of *N*-sulfonyl imidate esters derived from various model sulfonamides as well as from carbonic anhydrase inhibitors were examined in aqueous solution and in human plasma solutions. The hydrolysis of the compounds was subject to specific acid and base catalysis as well as enzymatic catalysis by plasma enzymes. At pH 4–8 or in the presence of plasma all the compounds derived from alcohols and amino alcohols were converted quantitatively to the parent sulfonamide and the corresponding carboxylic acid or carbonate ester. Sulfonyl imidate esters derived from phenols hydrolyzed to yield *N*-acyl sulfonamide and the parent phenol. The influence of various structural factors on the reactivity of the sulfonyl imidate esters was examined. The results suggest that the *N*-sulfonyl imidate esters are potentially useful prodrug forms containing a primary sulfonamide group as well as for carboxylic acid esters derived from aliphatic alcohols.

Introduction

Carbonic anhydrase inhibitors such as acetazolamide, ethoxzolamide and methazolamide are useful for the treatment of glaucoma. However, due to limited aqueous solubility or unfavourable lipophilicity (Fig. 1) they are not active when given topically to the eye and must be given orally or parenterally (Maren et al., 1983; Friedland and Maren, 1984; Eller et al., 1985; Maren, 1987). Systemic side effects severely limit this mode of therapy (Friedland and Maren, 1984) and consequently, great activities are presently going on to find a new carbonic anhydrase inhibitor that would readily penetrate the cornea and be active in lowering intraocular pressure when topically administered to the eye (Maren, 1987, and references cited therein).

An alternative approach to solve the delivery problems with these drugs, which all contain a primary sulfonamide group as the most prominent functional moiety, may be the development of prodrug derivatives possessing adequate water solubility and lipophilicity characteristics combined with the ability to be reconverted to the parent active sulfonamide following corneal passage. As a continuation of studies aiming at identifying bioreversible derivatives for the sulfonamide group (Larsen and Bundgaard, 1987; Larsen et al., 1988), we now report on the potential

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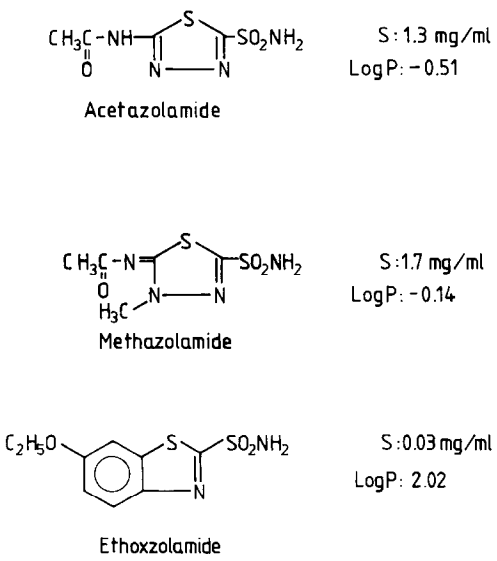
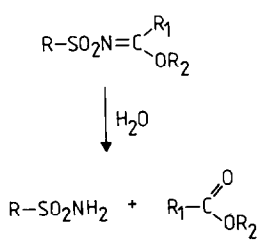


Fig. 1. Structures of the carbonic anhydrase inhibitors acetazolamide, methazolamide and ethoxzolamide. S refers to the solubility of the compound in aqueous buffer solution of pH 7.40 at 22 °C and P is the partition coefficient between octanol and 0.02 M phosphate buffer of pH 7.40.

applicability of *N*-sulfonyl imidate esters as a prodrug form. A series of *N*-sulfonyl imidate esters of *p*-toluenesulfonamide, used as a model sulfonamide, as well as of some carbonic anhydrase inhibitors were prepared and their kinetics and mechanism of degradation examined in aqueous solution and in human plasma solutions at 37 °C. As will be described below an interesting aspect of these derivatives is that they can also be considered as a prodrug type for carboxylic acid esters, in which case the sulfonamide component would act as the promoiety (Scheme 1). A part of this work has previously been described in a preliminary communication (Bundgaard and Larsen, 1988).



Scheme 1.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was done with a Kontron apparatus consisting of an LC pump T-414, a Uvikon 740 LC UV detector, a 20 μl loop injection valve and a Chrompack column (100 × 3 mm) packed with Chromspher C 18 (5-μm particles) and equipped with a guard column. 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 360 L instrument. IR spectra were taken on a Unicam instrument using the potassium chloride technique.

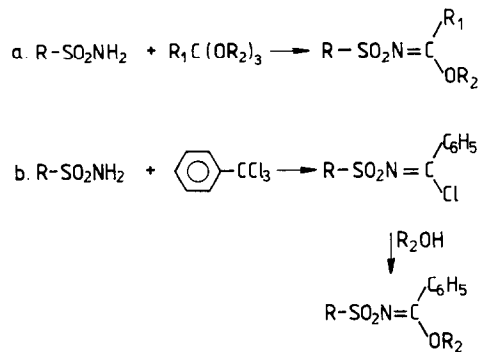
Measurements of pH were done at the temperature of study using a Radiometer Type PHM 26 instrument. Melting points were taken on a capillary melting point apparatus and are uncorrected. Microanalyses were performed at Leo Pharmaceuticals, Ballerup, Denmark or at the Microanalytical Department, University of Copenhagen.

Chemicals

Benzenesulfonamide, *p*-toluenesulfonamide, methanesulfonamide, 4-nitrobenzenesulfonamide and various orthoesters were commercially available. 2,4-Dinitrobenzenesulfonamide was prepared as described by Willgerodt and Mohr (1986) and ethoxzolamide as reported by Schoenwald et al. (1984). Methazolamide was obtained by extracting Neptazane tablets with acetone and recrystallized from acetone–hexane, m.p. 212–214 °C. *N*-Acylated *p*-toluenesulfonamides were obtained as previously described (Kemp and Stephen, 1948; Larsen and Bundgaard, 1987).

Synthesis of N-sulfonyl imidate esters

The *N*-sulfonyl imidate esters **1–6** (see Table 1 and **22–32** (see Table 2) were all prepared by reacting the parent sulfonamide with the appropriate orthoester (Scheme 2, reaction a) according to literature methods (Loev and Kormendy, 1964; Runti et al., 1960; Tosolini, 1961; Yale and Sheehan, 1961). A typical procedure is given for



Scheme 2.

the preparation of compound **2**: a mixture of 4.25 g (25 mmol) of *p*-toluenesulfonamide and 8 ml of trimethyl orthoacetate was refluxed for 7 h. The resulting solution was concentrated in vacuo. The residue obtained crystallized upon trituration with ether and was recrystallized from ether to give 3.5 g of **2**. The compounds **20**, **21**, **28** and **29** were prepared in an analogous manner from tetramethyl or tetraethyl orthocarbonate as described for compound **21** by Meyer (1963). In the case of the ethoxzolamide derivatives **28** and **29** however, acetone was included in the reaction mixture for solubility reasons. Thus, compound **28** was obtained by refluxing a mixture of ethoxzolamide (1.0 g), tetramethyl orthocarbonate (8 ml) and acetone (6 ml) for 5 h. The solid deposited upon concentrating the reaction solution in vacuo was recrystallized from acetone to give 0.8 g of compound **28**.

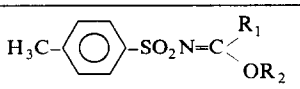
The remaining *N*-sulfonyl imidate esters all containing phenyl as the R_1-substituent (compounds **7–19**) were prepared by reacting *N*-(*p*-toluenesulfonyl)benzimidoyl chloride, obtained from *p*-toluenesulfonamide and phenyltrichloromethane as described previously (Dubina and Burmistrov, 1966), with the appropriate alcohol (compounds **7–12**), phenol (**13** and **14**) or amino alcohol (**15–19**) in acetone solutions in the presence (**7–14**) or absence (**15–19**) of pyridine or triethylamine (Scheme 2, reaction b). Typical procedures used (Dubina et al., 1969) are as follows: to a solution of *N*-(*p*-toluenesulfonyl)benzimidoyl chloride (1.17 g, 4 mmol) in acetone (4 ml) and pyridine (0.32 g, 4 mmol) was added the appropriate alcohol (5 mmol). The reaction solution was stirred at room temperature for 2 h and then diluted with about 30 ml of water. Upon standing overnight at 4°C the *N*-sulfonyl imidate ester initially separated as an oil crystallized. It was filtered off and recrystallized from acetone–petroleum ether or ethyl acetate–petroleum ether. This procedure was used for the preparation of compounds **7–13**. The 2-hydroxy-*N*,*N*-dimethylacetamide used for the synthesis of compound **11** was obtained as previously described (Nielsen and Bundgaard, 1988). Compound **14** was prepared as follows: a solution of *N*-(*p*-toluenesulfonyl)benzimidoyl chloride (1.17 g, 4 mmol) in acetone (4 ml) was added to a solution of the *N*,*N*-dimethylglycolamide ester of salicylic acid (Bundgaard and Nielsen, 1988) (0.83 g, 4 mmol) in acetone (8 ml) and triethylamine (0.56 ml, 4 mmol). The mixture was stirred at room temperature overnight, filtered and evaporated under reduced pressure. The residue obtained was taken up in ethyl acetate and crystallized by the addition of petroleum ether. By recrystallization from ethyl acetate 1.2 g of compound **14** was obtained.

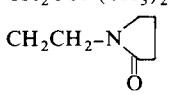
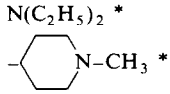
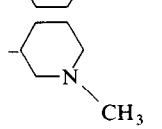
The procedure used for the preparation of the sulfonyl imidate esters containing an amino group (**15–19**) was as follows: a solution of the appropriate amino alcohol (4 mmol) in acetone (2 ml) was added dropwise to a solution of *N*-(*p*-toluenesulfonyl)benzimidoyl chloride (1.17 g, 4 mmol) in acetone (4 ml). The mixture was then stirred for 20 h at room temperature. Ether (20 ml) was added and after standing for 4 h at 4°C the sulfonyl imidate ester precipitated as the HCl salt was filtered off, washed with ether and recrystallized from ethanol–ether.

Melting points of the *N*-sulfonyl imidate esters are given in Tables 1 and 2. Elemental analyses (C, H and N) were in all cases within ±0.4% of the theoretical values. The ^{1}H NMR spectra of the derivatives were consistent with the structures. All derivatives showed a strong bond at 1600–1620 cm^{-1} in their IR spectra which is typical for the C=N bond (Yale and Sheehan, 1961). Some of the compounds have been described before: **1** (Runti et al., 1960), **2** (Loev and Kormendy, 1964), **7–10** and **13** (Dubina et al., 1969), **21** (Meyer, 1963) and

TABLE 1

Physical and hydrolysis data for various N-sulfonyl imidate esters



Compd.	R_1	R_2	m.p. (°C)	Rate parameters at 37 °C		
				k_0 (min^{-1})	k_H ($M^{-1}min^{-1}$)	k_{OH} ($M^{-1}min^{-1}$)
1	H	C_2H_5	51–53	0.6	95	4.3×10^5
2	CH_3	CH_3	74–75	4.7×10^{-3}	36	2.7×10^3
3	C_3H_7	CH_3	42–44	–	35	3.1×10^3
4	C_4H_9	CH_3	~ 25	–	38	3.0×10^3
5	CH_3	C_3H_5	52–53	3.6×10^{-3}	30	2.0×10^3
6	C_2H_5	C_2H_5	43–44	–	93	3.4×10^3
7	C_6H_5	CH_3	123–124	–	2.4	1.1×10^3
8	C_6H_5	C_2H_5	74–75	3.4×10^{-1}	3.8	7.1×10^2
9	C_6H_5	C_3H_7	86–87	–	3.9	5.7×10^2
10	C_6H_5	*i*-C_3H_7	73–74	4.7×10^{-3}	1.4	1.9×10^2
11	C_6H_5	$CH_2CON(CH_3)_2$	166–167	–	0.7	7.5×10^2
12	C_6H_5		106–107	–	2.6	1.6×10^3
13	C_6H_5		158–159	1.3×10^{-2}	–	–
14	C_6H_5		164–165	7.4×10^{-3}	0.08	5.3×10^3
15	C_6H_5	$CH_2CH_2N(CH_3)_2$ *	144–145	2.1×10^{-2}	0.3	1.0×10^6
16	C_6H_5	$CH_2CH_2CH_2N(CH_3)_2$ *	156–157	1.3×10^{-2}	1.9	1.0×10^5
17	C_6H_5	$N(C_2H_5)_2$ *	134–135	2.1×10^{-2}	0.5	1.0×10^6
18	C_6H_5	 *	165–167	7.9×10^{-3}	0.7	2.9×10^2
19	C_6H_5		154–156	7.1×10^{-3}	0.1	3.3×10^2
20	OCH_3	CH_3	149–152	7.4×10^{-4}	0.18	2.8×10^3
21	OC_2H_5	C_2H_5	70–71	1.4×10^{-4}	0.05	5.1×10^2

* HCl salt.

25 (Barber, 1943). The melting points observed for these compounds agreed with those reported.

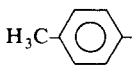
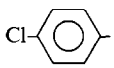
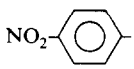
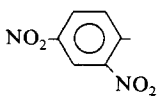
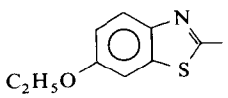
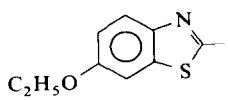
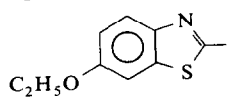
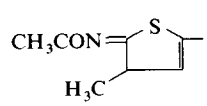
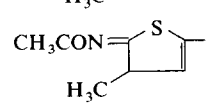
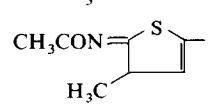
Kinetic measurements

The degradation of the *N*-sulfonyl imidate esters was studied in aqueous buffer solutions at 37.0 ± 0.2 °C. Hydrochloric acid, acetate, phosphate, borate, carbonate and sodium hydroxide buffers were used; the total buffer concentration was generally 0.01 M and a constant ionic strength (μ) of 0.5 was maintained, when possible, for each buffer by adding a calculated amount of potassium chloride.

The rates of hydrolysis were in some cases (at basic pH) followed by recording the absorbance change accompanying the hydrolysis at 230–250 nm where the change was maximal. The reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quarz cuvette and were initiated by adding 25 μl of stock solu-

TABLE 2

Physical and hydrolysis data for various N-sulfonyl imidate esters

Compd.	$R-SO_2N=C(R_1)(OR_2)$ R	R_1	R_2	m.p. (°C)	Rate parameters at 37 °C k_0 (min^{-1})	k_H ($M^{-1}min^{-1}$)	k_{OH} ($M^{-1}min^{-1}$)
22	CH_3	C_6H_5	C_2H_5	61–62	–	2.8	6.5×10^2
23		C_6H_5	C_2H_5	58–59	5.0×10^{-3}	2.9	1.1×10^3
8		C_6H_5	C_2H_5	74–75	3.4×10^{-3}	3.8	7.1×10^2
24		C_6H_5	C_2H_5	72–73	9.3×10^{-3}	2.5	1.2×10^3
25		C_6H_5	C_2H_5	129–130	3.6×10^{-2}	1.1	2.4×10^3
26		C_6H_5	C_2H_5	164–165	1.8×10^{-2}	0.35	3.0×10^2
27		C_6H_5	C_2H_5	124–125	9.1×10^{-3}	0.10	2.2×10^2
28		OCH_3	CH_3	165–166	3.4×10^{-2}	–	2.7×10^4
29		OC_2H_5	C_2H_5	105–106	8.3×10^{-3}	–	7.2×10^3
30		H	CH_3	150–151			
31		CH_3	CH_3	109–110			
32		CH_3	C_2H_5	105–106			

tions of the derivatives in acetonitrile or in water (**15–19**) to give a final concentration of 10^{-5}–10^{-4} M. Pseudo-first-order rate constants were determined from the slopes of linear plots of log $(A_t - A_\infty)$ or log $(A_\infty - A_t)$ against time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

Most reactions were followed by using reversed-phase HPLC methods capable of separating the imidate esters and their products of hy-

drolysis. Mobile phase systems of 40–60 v/v methanol in 0.01 M acetate buffer of pH 4.0 were generally used. For compound **14** a mobile phase consisting of 45 v/v methanol in a 0.02 M phosphate buffer of pH 7.0 was used. Analysis of the products formed upon hydrolysis required in general the use of eluting systems with a lower content of methanol. The flow rate was 0.5–1.2 ml min^{-1} and the column effluent was monitored 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. The reactions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile, ethanol or water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentrations of the compounds being 2×10^{-6} to 2×10^{-4} M. The solutions were kept in a water-bath at 37° C and at appropriate intervals samples were taken and chromatographed immediately. In some cases (very fast reactions) the samples taken were neutralized with hydrochloric acid or sodium hydroxide solutions to stop the reaction before the chromatographic analysis. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual derivative against time or, in the cases of compounds **15–19**, from the amounts of parent sulfonamide produced upon hydrolysis.

The degradation of the very unstable methazolamide derivatives **30–32** was followed by monitoring the appearance of methaxzolamide using a solvent system of methanol and 0.02 M acetate buffer pH 4.0 (1 : 3 v/v); the wavelength for detection was 280 nm.

Hydrolysis in human plasma solutions

The hydrolysis of the derivatives was studied in 0.01 M phosphate buffer (pH 7.4) containing 80% human plasma at 37° C. At appropriate times, samples of 250 μl of the plasma solutions with an initial concentration of the derivatives of 10^{-5}–10^{-4} M were withdrawn and deproteinized by mixing with 1000 μl of methanol or 500 μl of a 2% solution of zinc sulphate in methanol–water (1 : 1 v/v). After centrifugation for 2 min at 13,000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of hydrolysis

The kinetics of decomposition of the *N*-sulfonyl imidate esters was determined in aqueous buffer solutions of various pH values at 37° C. At constant pH and temperature the reactions displayed strict first-order kinetics over several half-lives whether the reactions were monitored for loss of imidate ester or appearance of the parent sulfonamide. Some typical first-order plots are shown in Figs. 2 and 3. Except for compounds **20**, **21**, **28** and **29** the sulfonyl imidates can exist as Z or E isomers. The exact configuration of the compounds is not known. The good linear first-order kinetics observed indicate, however, that if a compound is a mixture of Z and E isomers, the reactivity of these does not differ significantly. Biphasic first-order kinetics has previously been observed for the hydrolysis of phenyl *N*-methylacetimidates and ascribed to different reactivities of the E and Z forms as well as interconversion of the isomers (Satterthwait and Jencks, 1974 b). At the buffer concentrations used (0.01 M) no significant buffer catalytic effect was observed.

The pH–rate profiles for all the derivatives studied were U-shaped as illustrated in Figs. 4–6, indicating that the degradation of the compounds

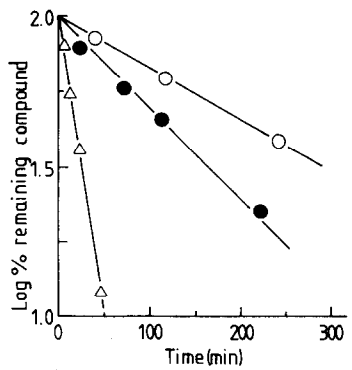


Fig. 2. Plots showing the first-order kinetics of degradation of compound **8** in aqueous buffer solutions (at 37° C) of pH 3.0 (●), pH 7.4 (○) and pH 9.4 (△).

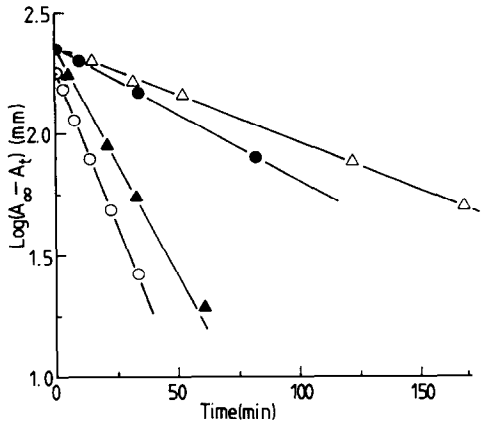


Fig. 3. Plots showing the first-order kinetics of degradation of compound **18** in aqueous buffer solutions (at 37 °C) of pH 1.1 (○), pH 2.1 (●), pH 7.4 (△) and pH 9.4 (▲), A_∞ and A_t refer to the amount of *p*-toluenesulfonamide formed at infinity and at time *t*, respectively.

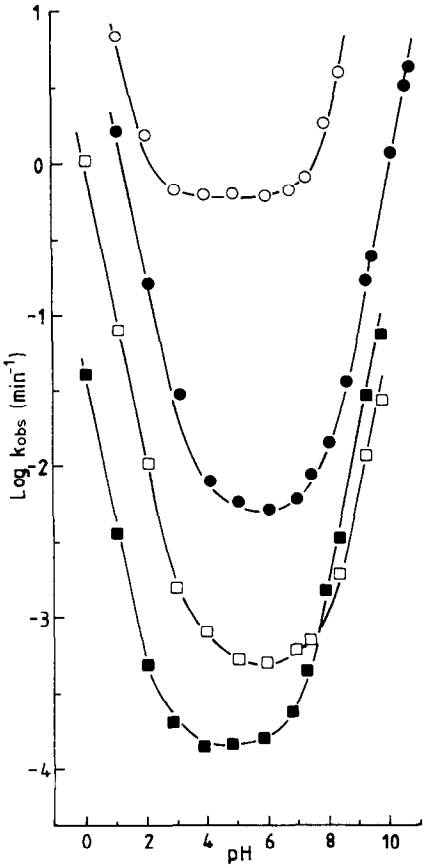


Fig. 4. The pH–rate profiles for the degradation of the *N*-sulfonyl imidate esters **1** (○), **2** (●), **10** (□) and **21** (■) in aqueous solution ($\mu = 0.5$) at 37 °C.

can be described in terms of specific acid-catalyzed (k_H), spontaneous (k_0) and specific base-catalyzed (k_{OH}) reactions according to the following equation:

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

where a_H and a_{OH} are the hydrogen ion and hydroxide ion activity, respectively, and k_H and k_{OH} are second-order catalytic rate constants. The hydroxide ion activity was calculated from the measured pH according to Eqn. 2 (Harned and Hamer, 1933):

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

The values of the rate parameters k_H, k_{OH} and k_0 are listed in Tables 1 and 2. The stability of the methazolamide derivatives **30–32** was so limited ($t_{1/2} < 1$ min at 37 °C at all pH values) that no specific rate constants were obtained.

The pH–rate profiles (Fig. 5) obtained for the *N*-sulfonyl imidate esters containing an amino function (**15–19**) show a levelling off of the rate with increasing pH in the alkaline pH region. This

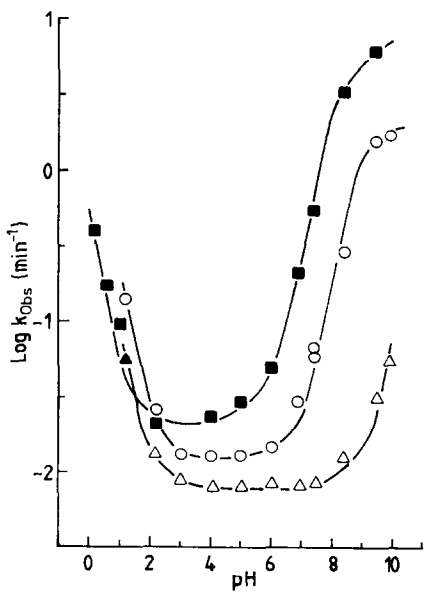


Fig. 5. The pH–rate profiles for the degradation of the *N*-sulfonyl imidate esters **16** (○), **17** (■) and **18** (△), derived from amino alcohols, in aqueous solution ($\mu = 0.5$) at 37 °C.

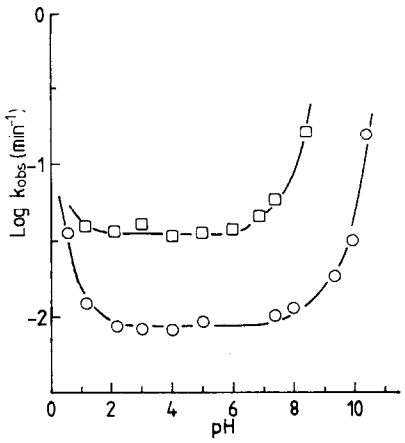


Fig. 6. The pH–rate profiles for the degradation of the ethozolamide derivatives **27** (○) and **28** (□) in aqueous solution ($\mu = 0.5$) at 37 °C.

can be ascribed to different reactivities of the protonated and free base forms of the compounds in analogy with the hydrolysis of carboxylic acid esters of amino alcohols (e.g. Bruice and Mautner, 1973; Bundgaard et al., 1986). The k_{OH}-values listed in Table 1 for these imidate esters were calculated from the rate data at neutral and slightly basic pH values.

Maximal stability of the imidate esters was generally achieved at pH 4–6. The half-lives of hydrolysis at pH 6 and pH 7.4 are listed in Table 3 along with the half-lives of decomposition in 80% human plasma solutions. As appears from the rate data obtained, hydrolytic enzymes in plasma markedly accelerate the rate of hydrolysis. The plasma-catalyzed hydrolysis is particularly marked for compound **11** which is in accordance with the very facile plasma-catalyzed hydrolysis recently described for *N*,*N*-dimethylglycolamide esters of various carboxylic acids (Bundgaard and Nielsen, 1987, 1988; Nielsen and Bundgaard, 1988). Enzymatic hydrolysis of imidate esters does not appear to have been reported before.

Structural effects influencing reactivity

As is the case for carboxylic acid esters electronic and steric effects within the substituents R_1 and R_2 influence the rate of hydrolysis of the *N*-sulfonyl imidate esters. The polar effects of the R_2 alkyl groups in compounds **7**–**10** are almost identical and the observed differences in reactivity in neutral and alkaline solutions can be ascribed to differences in the steric properties as shown in Fig. 7, where log k_{OH} is plotted against the steric substituent parameter ν (Charton, 1977). Sterically hindered substituents as the *i*-propyl group markedly decrease the rate of hydrolysis as well as the ease of enzymatic hydrolysis. The formimidate ester **1** is more reactive than the corresponding alkyl imidates **5** and **6** which is analogous to the behaviour of formate esters relative to alkyl esters.

TABLE 3

Half-lives of hydrolysis at 37 °C of various N-sulfonyl imidate esters

Compd.	$t_{1/2}$ (min)		
	pH 6.0	pH 7.4	80% human plasma [b]
1	1.2	0.8	<1
2	150	83	8.1
3	–	145	32
4	–	160	28
5	175	144	6.4
6	–	108	24
7	–	114	7.0
8	220	175	23
9	–	175	20
10	1380	1045	195
11	–	144	2.0
12	–	75	4.8
13	–	55	–
14	93	91	0.6
15	35 [a]	2.7	<1
16	53 [a]	11	4
17	35 [a]	1.3	<1
18	90	90	36
19	98	73	25
20	870	255	32
21	4400	1580	78
22	–	354	44
23	139	124	9.9
24	75	64	4.4
25	19	18	0.8
26	39	35	0.3
27	76	70	0.1
28	20	12	<1
29	84	63	<1
31	1.3 [c]	<1	<1

[a] At pH 4.0 (pH minimum of hydrolysis).
[b] Human plasma diluted to 80% with 0.01 M phosphate buffer of pH 7.40.
[c] At 24 °C.

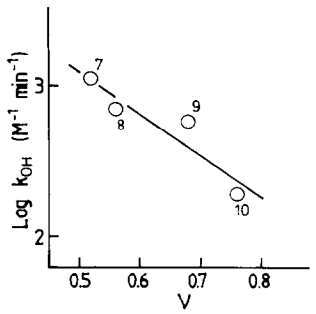


Fig. 7. Plot of log k_{OH} vs the steric parameter (ν) for the *N*-sulfonyl imidate esters **7–10**. The ν values refer to the R_2 moiety.

The imidate esters derived from amino alcohols (**15–19**) are more reactive than the other esters which most likely is due to intramolecular assistance by the amino group coupled with the high polar effect of a protonated amino group. In analogy with the hydrolysis of carboxylic acid esters of similar amino alcohols (Hansen, 1962; Ågren et al., 1961; Aksnes and Frøyen, 1966; Bruice and Mautner, 1973) this assistance may be attributed to either intramolecular general acid-catalyzed hydroxide ion attack or intramolecular general base catalysis by the unprotonated amino group of water attack on the ester group (Scheme 3). In compounds **18** and **19** the amino function is placed in a position that makes it unable to exhibit such intramolecular catalysis, and the stability of these compounds is somewhat higher than that of the imidates **15–17**.

Scheme 3.

Considering the influence of the sulfonyl part on reactivity, compounds **8** and **22–27** listed in Table 2 are useful to compare in that they have the same R_1 and R_2 substituents. Whereas the reactivity in acidic solutions does not differ appreciably there is a large difference in the stability in neutral and alkaline solutions. Fig. 8 shows a plot of log k_{OH} and log k_0 against the pK_a value of the parent sulfonamides. Surprisingly, no linear relationships are observed. Instead, the data appear to indicate a maximal reactivity occurring for

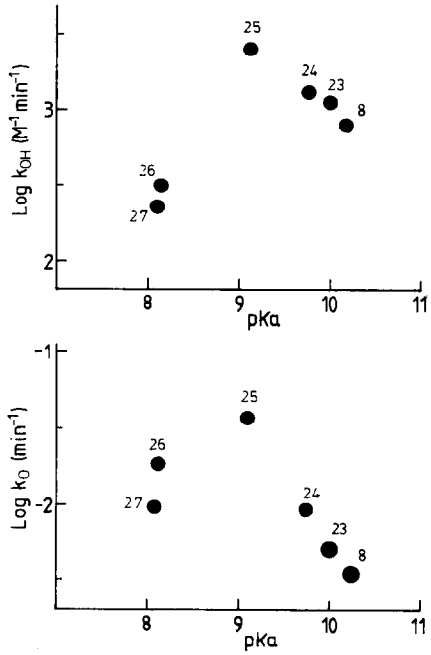


Fig. 8. Plots of log k_{OH} and log k_0 vs the pK_a of the parent sulfonamide for various *N*-sulfonyl imidate esters. The following pK_a values were used (all at 20 °C): 10.17 (*p*-toluenesulfonamide), 10.00 (benzenesulfonamide), 9.77 (4-chlorobenzenesulfonamide), 9.14 (4-nitrobenzenesulfonamide) (all taken from Willi (1956)), 8.14 (2,4-dinitrobenzenesulfonamide) (present study) and 8.12 (ethoxzolamide) (from Eller et al. (1985)).

a sulfonyl imidate ester with a pK_a of about 9 for the parent sulfonamide. This behaviour is difficult to explain, the more so as the ethoxzolamide derivative **28** is more reactive than the corresponding compound **20** derived from *p*-toluenesulfonamide whereas the opposite is the case for compounds **27** and **8**. Steric effects may probably play a role besides the polar effect of the sulfonamide group as considered in terms of pK_a values. The general trend of increased reactivity with increasing acidity of the parent sulfonamide is, however, underlined by the very high instability (Table 3) of the sulfonyl imidate esters (**30–32**) derived from methazolamide which has a pK_a value of 7.4 (Maren et al., 1983).

Products and mechanism of hydrolysis

Previous investigations (Dubina et al., 1969; Okuyama et al., 1973a) have shown that *N*-sulfonyl imidate esters may hydrolyze to give sulfonamide

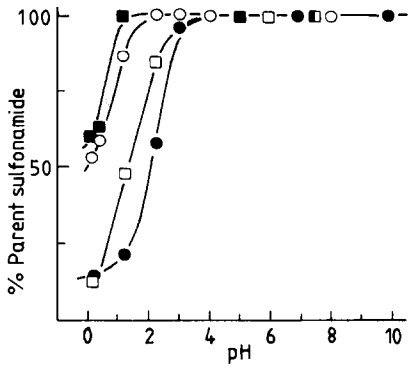


Fig. 9. The effect of pH on the yield of parent sulfonamide formed upon hydrolysis of the *N*-sulfonyl imidate esters **8** (●), **15** (○), **24** (□) and **26** (■) in aqueous solution at 37 °C.

and ester or *N*-acyl sulfonamide and alcohol. The identity of the products formed upon hydrolysis of compounds **1–32** was verified by HPLC analysis by comparison with the parent sulfonamide and the corresponding *N*-acyl sulfonamides and esters. In agreement with earlier findings reported for the hydrolysis of compound **2** (Okuyama et al., 1973a) the product distribution was found to vary with pH of solution (Figs. 9 and 10). At pH < 3–4 the compounds degraded to yield a mixture of sulfonamide and *N*-acyl sulfonamide whereas at pH > 4 the hydrolysis was found to proceed with the quantitative formation of sulfonamide and the corresponding ester in most cases. The exceptions were compounds **13** and **14** which hydrolyzed exclusively to *N*-(benzoyl)-*p*-toluenesulfonamide and the parent phenol independent of the pH of

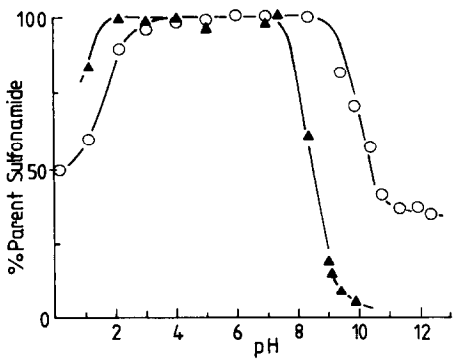
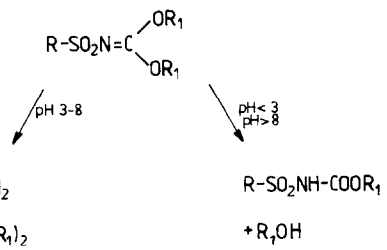


Fig. 10. The effect of pH on the yield of parent sulfonamide formed upon hydrolysis of compound **20** (○) and **28** (▲) in aqueous solution at 37 °C.

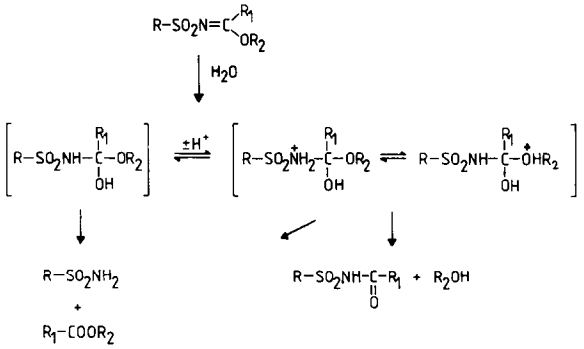
solution as well as in the presence of plasma. Compounds **13** and **14** differ from the other sulfonyl imidates by being derived from phenols instead of alcohols and the differential behaviour in the products of degradation may be ascribed to the better leaving ability of a phenol compared with an alcohol. In the case of the compounds **20**, **21**, **28** and **29** a further shift in the product distribution with pH was observed. As can be seen from Fig. 10 the parent sulfonamide (and the corresponding dialkyl carbonate esters) was only formed in quantitative amounts in the pH range from about 3.5 to about 8. Outside this pH range the major degradation product formed was the corresponding *N*-alkoxycarbonyl sulfonamides (Scheme 4). Of essential importance for the consideration of sulfonyl imidate esters as prodrugs for sulfonamides is the fact that all the compounds except those derived from phenols (**13** and **14**) hydrolyzed to give the parent sulfonamide in quantitative amounts at physiological pH (7.4) as well as in the presence of human plasma. The latter observation shows that the plasma-catalyzed hydrolysis is in fact a catalysis of the reaction shown in Scheme 1. As reported previously (Larsen and Bundgaard, 1987), *N*-acylated primary sulfonamides are chemically very stable and also rather resistant to enzymatic hydrolysis by plasma.



Scheme 4.

According to previous investigations (Okuyama et al., 1973a and b; Smith and Schmir, 1975; Satterthwait and Jencks, 1974a) on the hydrolysis of imidate esters the degradation proceeds through rate-limiting formation of tetrahedral addition intermediates being in acid–base equilibrium (Scheme 5).

At low pH the protonated intermediate decomposes mainly by expulsion of R_2OH with the formation of the stable *N*-acyl sulfonamide



Scheme 5.

whereas at higher pH values the neutral intermediate expels sulfonamide anion (pK_a 10.2 for *p*-toluenesulfonamide (Willi, 1956)) in preference to alkoxide ion (pK_a 15–16 (Ballinger and Long, 1960)). When the acidity of R_2OH becomes equal to or greater than that of the sulfonamide as in the case of phenols (pK_a 9–10) the reverse expulsion may become favoured. Accordingly, a sulfonyl imidate ester may expectedly serve as a prodrug for a sulfonamide (or a phenolate ester) if the sulfonamide has a pK_a lower than that of the phenol. For compounds **13** and **14**, this requirement is not met and no sulfonamide or ester is formed upon their hydrolysis as described above.

Conclusions

The results obtained show that *N*-sulfonyl imidate esters satisfy several criteria for functioning as prodrug forms for compounds containing a primary sulfonamide group. The imidate esters derived from alcohols are converted quantitatively to the parent sulfonamide at physiological conditions of pH and temperature, and since the hydrolysis is plasma-catalyzed a high rate of prodrug conversion is expected in vivo. By varying the ester portions of the derivatives it is readily possible to control such physicochemical properties as water solubility and lipophilicity of importance for prodrug absorption. Thus, by introducing an ionizable amino function in the alcohol portion, *N*-sulfonyl imidate esters with a high water solubility can be obtained. As hydrochloride salts the compounds **15**–**19** have a water solubility greater than 10%. A drawback of the derivatives is, however, their limited chemical stability in aqueous solution. The esters are most stable at weakly acidic pH values but even at this pH the stability is so limited that aqueous solutions, e.g. eye-drop formulations of derivatives of carbonic anhydrase inhibitors, with practical shelf-lives cannot be made. Formulation into polymeric matrices or the use of ophthalmic rods (Gwon et al., 1986) may eventually be useful to overcome the instability problem.

As pointed out previously (Bundgaard and Larsen, 1988), *N*-sulfonyl imidates may equally well be considered as a prodrug type for carboxylic acid esters, in which case the sulfonamide component acts as the promoiety. Numerous drugs contain an ester group as an essential part of their structure, e.g. various calcium antagonists, anticholinergic agents and steroid derivatives. Hitherto, no bioreversible derivatives have been explored for the ester functionality (Bundgaard, 1985). Studies are in progress to examine in more detail the potential applicability of *N*-sulfonyl imidates as prodrug forms for various drugs containing a carboxylic acid ester function.

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