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Kinetic and mechanistic studies on sulfamate esters: Models of enzyme inhibitors†

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ABSTRACT: Many compounds containing a sulfamate moiety, such as NH_2SO_2O— are now known to be medicinally important. However, very little is known about their mechanisms of reaction even under non-biological conditions. In this work the various types of elimination mechanisms that may occur have been probed by studying the kinetics of the reactions of model sulfamate substrates with amines (bases) that act as models for the enzymes involved. The principal mechanistic tool employed has been Brönsted plots and both 'normal' rectilinear and two types of biphasic plots have been found for the decomposition of the esters in acetonitrile (ACN). The mechanisms operating are seen as being of the E2 and E1cB types. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: sulfamate esters; 667-Coumate; emate; Brönsted biphasic plots; aminolysis mechanisms; enzyme inhibitors

INTRODUCTION

Sulfamate esters have been used as herbicides, sweeteners and pharmacological intermediates for many years. In recent years interest in compounds that contain the sulfamate functional group has increased enormously as awareness has risen of their important medical applications.[1]

Sulfamic acid **1** itself, the parent of the sulfamate moiety, possesses pharmacological applications. Its *O*-substituted derivatives **2**, *N*-substituted compounds **3**, and the *O,N*-di/trisubstituted compounds **4** and **5** respectively, are all biologically active (Scheme 1).[1]

Compound **1** is well known as a standard strong acid and both it and its salts are extensively used in the electrochemical industry. It is itself a potent inhibitor of the zinc enzyme, carbonic anhydrase (CA), which regulates the reversible hydration of carbon dioxide to bicarbonate. The *O*-substituted derivatives **2** have shown good inhibitory activity against unrelated enzymes such as the CAs and the steroid sulfatases (STSs), which regulate the formation of estrone from estrone sulfate. The *N*-substituted compounds **3**, have found application as sweeteners,[2] whilst the *O,N*-di/trisubstituted compounds **4** and **5** show a wide range of biological activities.[1] In this study it is the *O*-substituted derivatives **2**, the sulfamate esters, in which we are particularly interested, as these compounds have been shown to inhibit various unrelated enzymes. There are several drugs already in the market or in clinical trials containing the sulfamate moiety (Fig. 1).

Topiramate **6** is an anti-epileptic drug which is fast and effective and in clinical use at present. [3] Although the mechanism of action is not yet fully understood,[4] the sulfamate moiety has been shown to be crucial for this type of biological action.[3] An interesting side effect of **6** was that it caused weightloss in some patients, and thus it is now under investigation as an anti-obesity drug.[1] This development is exciting due to the widespread and increasing problem of obesity in the Western world. Avasimibe **7** is an anti-atherosclerotic sulfamate, restricting loss of elasticity of, and arterial wall thickening. It works by inhibiting acyl coenzyme A:cholesterol acyltransferase (ACAT).[1] This was a breakthrough discovery in 1994 and is now in clinical phase III trials, showing low toxicity and low incidence of side effects.[5] There are many other sulfamate drugs under investigation as anti-arthritic and anti-cholesterol agents. With the increasing number of bacteria resistant to traditional antibiotics and the increasing threat of HIV, AIDS Hep B and C etc., there is also a great amount of research ongoing in the development of antibiotic and antiviral sulfamates.[1]

The particular area of interest to this work is that of anticancer sulfamates. There are two different inhibitory activities of interest, that of STS inhibitors and that of CA inhibitors. STS is responsible for the hydrolysis of alkyl and aryl steroidal sulfamates therefore having a pivotal role in regulating the formation of biologically active steroids. These steroids support the growth of hormone-dependent cancers such as breast and prostate cancers. The enzyme inhibiting properties of these type **2** sulfamates prompted

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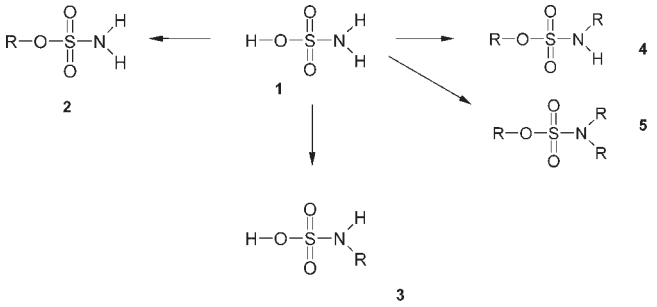


Scheme 1

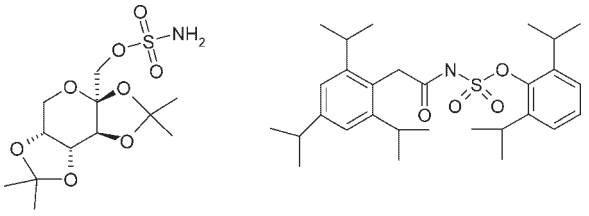


Figure 1. Examples of drugs in clinical use **(6)** and in trials **(7)**, which contain the sulfamate moiety

investigation to inhibit STS.[6] It was found that the pK_a of the sulfamate moiety had an effect on the potency of the inhibitor, with more acidic compounds being stronger inhibitors.[7] There are several steroidal and non-steroidal STS inhibitors now available with some of the most exciting discoveries in recent years coming from the Potter and Reed groups.[8] 667-Coumate **8** (Fig. 2) is currently in phase I clinical trials for treating post-menopausal women with breast cancer. An important feature is that it is non-estrogenic. Estrogen and estrogen mimics in the body promote hormone-dependent tumours.

CA isozymes, IX and XII are overexpressed in tumours and are usually associated with bad prognosis, as they render the tumour untreatable by radio- or chemotherapy. The series of first generation STS inhibitors were then tested for CA IX and XII inhibitory activity and recently Emate **9** has been confirmed as a potent inhibitor. Although emate is estrogenic and therefore unable to be developed for clinical use, its discovery allows for a new rationale in drug design.[9]

Work in our laboratory involves use of simple sulfamate esters as models of the drugs, and amines as models for the enzymes. Kinetic studies have been carried out in an attempt to elucidate the mechanism of action between amine and ester. Basically, the mechanism of the inhibitory process is not known though a few suggestions have been made. It is hoped that an understanding of the mechanism(s) will lead eventually to the design of better and more efficacious drugs.

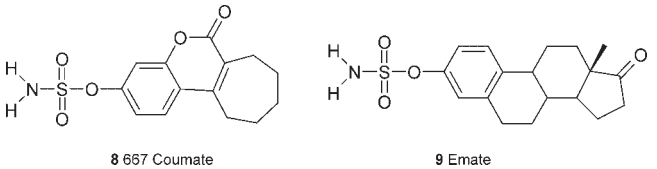


Figure 2. 667-Coumate, an STS inhibitor in clinical trials, and Emate, a potent CA inhibitor

EXPERIMENTAL

The sulfamate esters, RHN-SO_2-*O*-(C_6H_4)-NO_2-*p* **10** (R = CH_3 **a**, R = $PhCH_2$ **b**, and R = H **c**) were prepared. **10a** and **b** were prepared in earlier work in this laboratory.[10] For preparation of **10c** the method of Wong *et al.*[11] was used. All reagents were used as obtained from Aldrich unless otherwise specified. A stirred mixture of *p*-nitrophenol (1 equivalent) in DMA (1.5 mL per mmol *p*-nitrophenol) was cooled on ice. Pre-prepared sulfamoyl chloride (2 equivalents) was added slowly. The mixture was then stirred at room temperature and the reaction monitored by TLC using an 80:20 diethyl ether:pet. ether (40–60°C) eluent. When the reaction was judged complete the mixture was poured onto cold brine. The product was then extracted with three aliquots of ethyl acetate totaling 10–20 times the volume of DMA. This was then washed exhaustively with brine (typically five to six times using a volume equal to that of ethyl acetate each time). It was then dried over $MgSO_4$ and concentrated under reduced pressure. This method gave near quantitative yields and the crude product was purified if necessary by column chromatography using a short column of activated silica, 230–400 mesh, eluting with diethyl ether:pet. ether (40–60°C) in a stepwise manner from 20:80 to 100:0. The melting point range for **10c** is 107.2–111.8°C.

^{1}H NMRδ : (ppm):6.928 and 8.117(CH_2′*s on phenyl ring*), 9.642 and 11.086 (NH_2, sym and assym)

The kinetic method used for the three esters **10a**, **b**, and **c** was the same. HPLC grade acetonitrile (ACN) was obtained from Aldrich or Fischer and the water content was shown to be (0.02–0.07%) when tested by the Karl Fischer method. It was then used without further purification. Liquid amines were distilled under reduced pressure using a Kugelrohr where necessary. Solids were recrystallised from appropriate solvents. Stock solutions of ester were prepared in ACN with concentrations typically around 1×10^{-2} M. Stock solutions of each amine were prepared in ACN, typically of the order of 1–0.1 M. Aliquots of the amine solutions were then added to quartz cuvettes and made up to 2 mL using a micropipette with range 0.01–1.00 mL. The amine concentration in these solutions was 50- to 1000-fold in excess of the concentration of ester. These solutions were pre-heated in the cuvettes to the desired temperature. Reactions were initiated by injecting 0.02 mL ester (1×10^{-4} M) to the cuvettes containing amine solution using a micropipette. The cuvette that was stoppered, was then inverted to mix

and data collection was commenced immediately. Reactions were monitored by following the increase of the *p*-nitrophenol or *p*-nitrophenoxide ion absorbance. Aminolysis reactions were followed using a Cary 3E UV/vis spectrophotometer or Cary 50 UV/vis spectrophotometer. These were thermostatically controlled and fitted with multiple cell holders and sample transport at single wavelengths for the Cary 3E and at a wavelength range for the Cary 50. The absorbance data were saved as comma delineated workbook files and converted to a text document to be analysed using an OLIS-KINFIT programme, or manipulated by Excel or Origin. Absorbance data at fixed time intervals were analysed using the OLIS-KINFIT programme. Alternatively pseudo first order rate constants (k_{obs}) could be calculated by plotting $\log(A_\infty - A_t)$ or $\log(A_t - A_\infty)$ against time. The *p*-nitrophenol or *p*-nitrophenoxide product of aminolysis has been confirmed quantitatively by comparison with a spiked solution. The second order rate constant (k_2) for each reaction is determined by plotting $\log(k_{obs})$ versus [amine]. Linear plots were obtained (slope = k_2). Any deviation in the y-intercept from zero would be indicative of some other reaction happening, perhaps hydrolysis. This was not noticed.

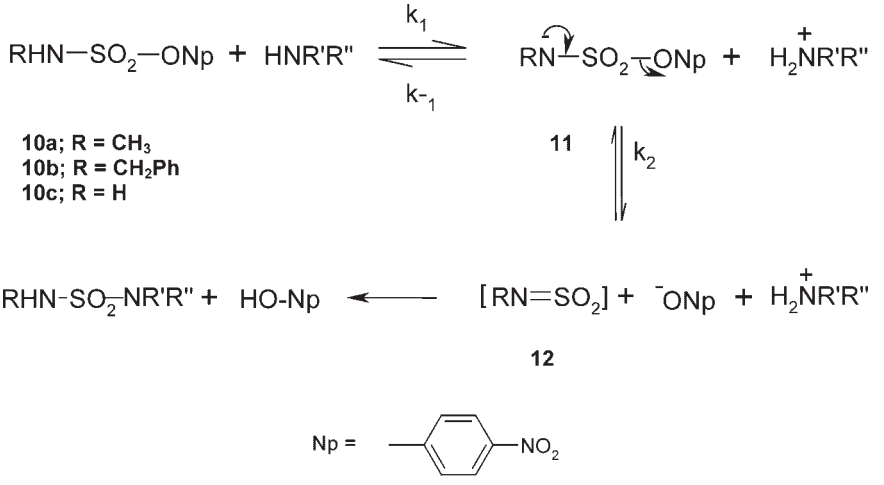
The pK_a's of **10a**, **b** and **c** were previously determined in this laboratory in ACN and found to be 17.9 and 17.7 and 17.8 respectively.[10]

RESULTS AND DISCUSSION

For almost 10 years we have been studying the aminolysis and hydrolysis of various sulfamate esters of type **10** (Scheme 2).[10,12–14] As the work progressed we have become more and more aware of the importance of certain of these materials in medicinal chemistry as outlined in the introduction above. We began to look on these sulfamate esters as models for the biologically active compounds and to regard the aminolysis reaction as a model for the enzymatic inhibition that occurs when compounds such as **6–9** are used. Thus our principal interest has been in the aminolysis rather than the hydrolysis reaction. In order to inhibit the hydrolysis reaction,[12] which is much more energetically favourable than the reaction with amines we have worked mainly in non-aqueous media ($CHCl_3$, ACN), principally ACN.

Following the important work many years ago of Williams and Douglas[15] who studied the hydrolysis and some aminolysis of **10a** an elimination-addition mechanism is favoured. The kinetics of these reactions and the present work were carried out under pseudo-first order conditions, that is, with a large excess of amine present, and reveal that reaction is first order in sulfamate ester and first order in amine. Therefore an E1 type mechanism involving spontaneous break-up of the ester is ruled out. An S_N2 mechanism at sulphur is very unlikely since Williams and Douglas[15] have shown that an ester which lacks a hydrogen on the nitrogen such as, $Me_2NSO_2OC_6H_4NO_2$-*p* and cannot therefore undergo elimination, reacts extremely slowly in aqueous organic media compared to esters of type **10**. In ACN, which has a much lower permittivity, we have been unable to get any reaction with this *N*, *N*-dimethyl ester.

Elimination mechanisms of the E2 and E1cB types are therefore favoured (Scheme 2). Sulfamate esters bearing a hydrogen on the nitrogen such as compounds **10** have pK_a values of ~8 in water-organic media and since they also have a good leaving group such as *p*-nitrophenol they seem to be pre-disposed towards elimination. The principal mechanistic tool we have used to explore the mechanisms operating in the reactions of these esters with amines has been Brönsted plots giving β_{nuc} values. Generally in $CHCl_3$ and ACN using various sets of amines (bases) such as pyridines, quinuclidines and alicyclic amines these have been straight line plots with slopes (β_{nuc}) varying from about 0.2–0.5 and have been interpreted as E2 mechanisms with varying degrees of Nβ—H bond cleavage (E1cB carbanionic character). Interpretation is aided by More O'Ferrall-Jencks type plots (Fig. 3).



Scheme 2

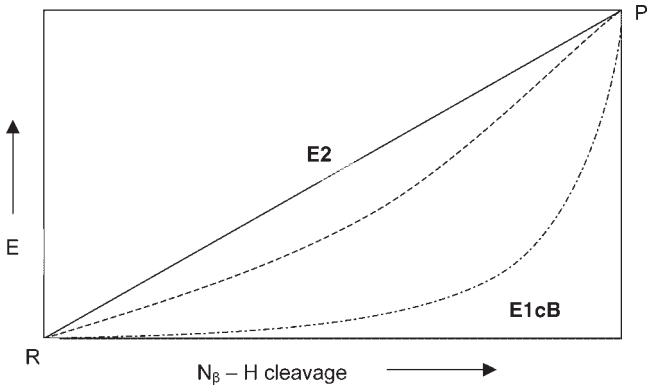


Figure 3. More O'Ferrall-Jencks diagram for aminolysis via E2 (——), E1cB (–·–·–) and E2 with E1cB character (– – –)

A few years ago we obtained interesting biphasic Brönsted plots using **10a** and **10b** in ACN with sets of alicyclic and pyridine bases (Figs. 4 and 5).[10] This type of effect has been observed by a number of groups for activated carbon substrates, such as $ArCH_2SO_2OAr$,[16,17] $XC_5H_5N^+CH_2CH_2Z$[18] and $NCCH_2CH_2SR$.[19] The mechanism of these reactions was interpreted as being E1cB with a change in the rate-determining step within the E1cB mechanism from $(E1cB)_{irrev}$ at the lower amine pK_as where $k_2 \gg k_{-1}[R^1R^2NH_2^+]$ to $(E1cB)_{rev}$ at the higher amine pK_as when $k_{-1}[R^1R^2NH_2^+] \gg k_2$. We feel that this interpretation is appropriate in the present case and thus at lower pK_a values (β_1 line) bimolecular formation of the conjugate base **11** of **10** (Scheme 2) occurs followed by rapid leaving group departure. With the stronger amines (β_2 line) departure of ONp from **10** is the slow step. The reaction may involve a very transient intermediate *N*-sulfonylamine **12**. In both cases the reaction products are *p*-nitrophenol and the appropriate sulfamide.

This change in rate-determining step seems to be strongly supported by the entropy changes occurring on changing from the β_1 region to the β_2 region. At the lower pK_as large negative entropies are observed indicating the bimolecular associative nature of the rate-determining step but, with the stronger amines where the Brönsted plot

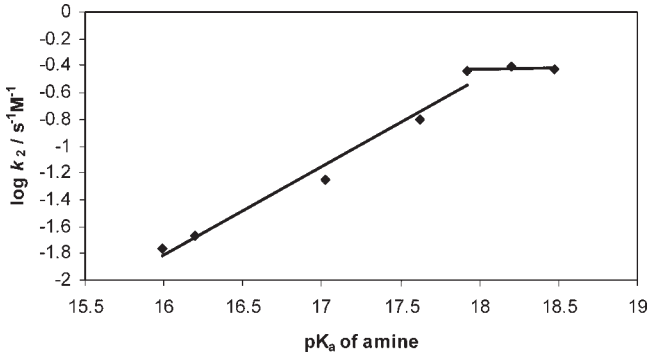


Figure 4. Brönsted biphasic plot for **10a** in ACN at 310 K for a series of alicyclic amines 'Reprinted with permission (*J. Org. Chem.* 2001, **66**, 6313–6316). Copyright (2001) American Chemical Society.'

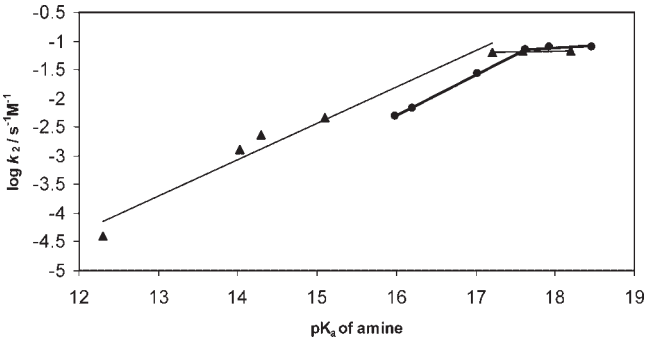


Figure 5. Brönsted biphasic plots for **10b** in ACN at 310 K for a series of alicyclic amines — and a series of pyridines — 'Reprinted with permission (*J. Org. Chem.* 2001, **66**, 6313–6316). Copyright (2001) American Chemical Society.'

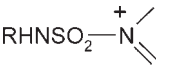
has virtually plateaued (β_2 region) and where general base catalysis is absent much less negative entropies have been measured pointing to unimolecular break-up of conjugate base **11**.

A few further points with regard to these reactions and particularly the biphasic plots may be made. It may be noted that the change in the rate-determining step seen in Figs. 4 and 5 occurs approximately at the point where the substrate pK_a is equal to that of the catalytic amine, that is

$$\Delta pK_a = pK_{R^1R^2NH_2} + -pK_a(\text{sulfamate}) \sim 0$$

The plots in Figs. 4 and 5 can be regarded as classical Eigen diagrams because the levelling off of the line arises at approximately ΔpK_a.

From Fig. 5 it is clear that the alicyclic amines react more rapidly with the substrates than the pyridines do and this is due to the fact that the former bases are stronger. If the reacting amine has a replaceable hydrogen then the sulfamide product will be as given in Scheme 2 but, if the amine, for example pyridine does not have a replaceable hydrogen than the product is of the type shown[20]



Recently we have transferred our attention to looking at the kinetics of aminolysis of compound **10c**. This

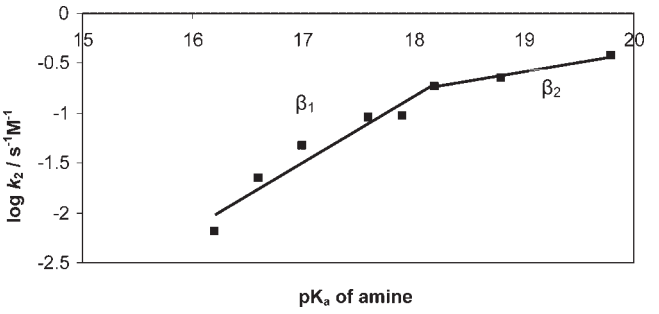


Figure 6. Brönsted biphasic plot for **10c** in ACN at 310 K for a series of alicyclic amines which are shown in (Fig. 7)

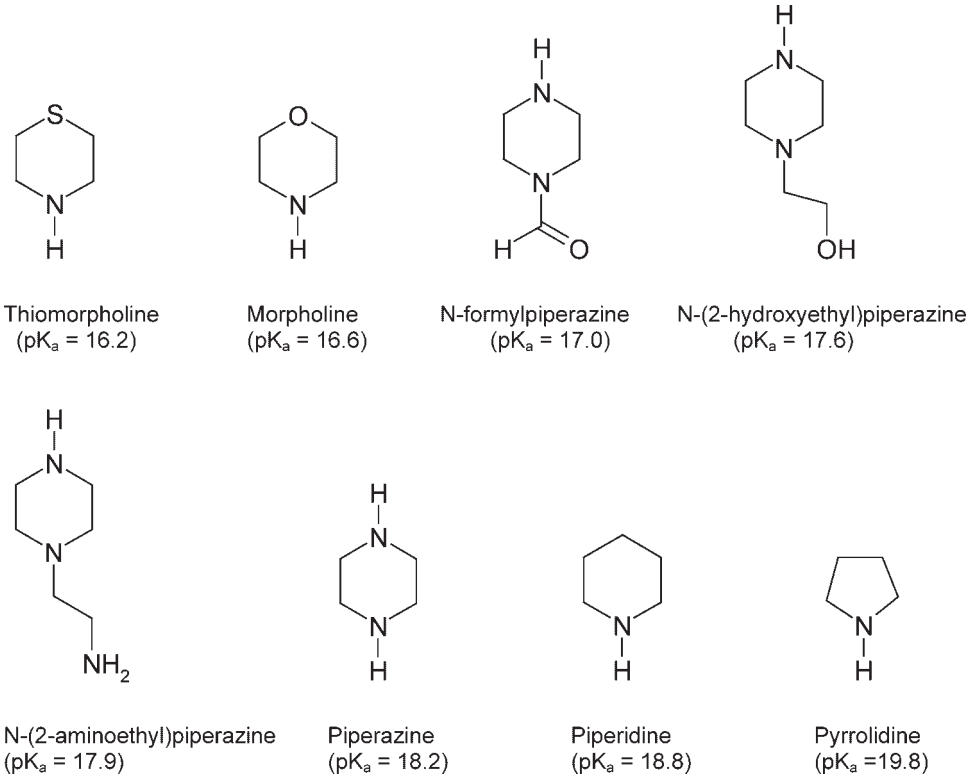
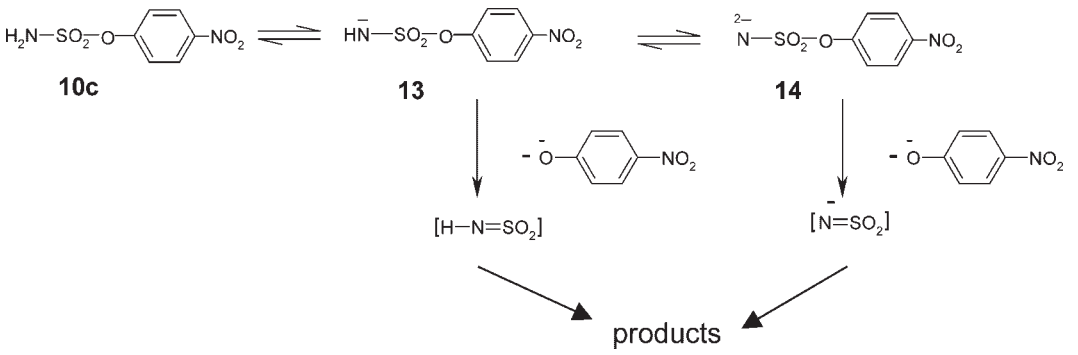


Figure 7. Series of alicyclic amines showing their pK_a in CAN

compound that possesses the simple sulfamate functionality, NH_2SO_2O— is closer in structure to 667-Coumate and emate and should therefore be a better model for these biological active materials. Figure 6 shows the Brönsted plot that was obtained using the eight secondary alicyclic amines illustrated in Fig. 7. The plot is again biphasic in character but obviously is different in that this time β_2 does not level off but continues to rise ($\beta_2 = 0.19$). Again the break point $pK_a \sim 18.2$ more or less corresponds to the pK_a of the ester **10c** (17.8).

This unusual behaviour can apparently be neatly explained using the scheme (Scheme 3) proposed by Thea and Williams and coworkers[21] to explain some years ago the kinetics of hydrolysis of **10c** in aqueous media. They proposed a double deprotonation leading first to the conventional *N*-sulfonylamine, [HN═SO_2] and at higher basic strength a novel anionic sulfonylamine, [$^-$N═SO_2]. Applying this kinetically supported scheme to our work in ACN the β_1 part of the Brönsted plot in Fig. 6 probably involves rate-determining removal by amine of the first proton giving **13** while when stronger bases are used the second proton will be removed to give the dianion, **14**. The product sulfamide and *p*-nitrophenol are of course the same in each case irrespective of which pathway is followed.

A pK_a of 7.3 measured spectrophotometrically in water has been reported[21] for the first ionisation and in ACN this value is 17.8 so the first ionisation must be substantially complete in ACN at this pK_a and formation of the dianion **14** then becomes important. When the second proton is removed one would expect to see a levelling off of the Brönsted plot (as was observed with **10b** and **10c**) but if



Scheme 3

the second pK_a is too high and beyond the pK_a range of the alicyclic amines that we have been using we will not see this levelling off. This is probably what is happening here. It has been suggested that the second pK_a in water for **10c** could be something like 15–16 and then as one might expect a value ~25 in ACN which would be well beyond the pK_a strengths of the bases employed here. Currently some stronger bases are being looked at to see if the β_2 line does level off.

In conclusion, our present results using the sulfamate esters **10** as models for the analogous sulfamate inhibitors, and aminolysis (with alicyclic amines and pyridines) as a model for the inhibition reaction, are very relevant for probing the mechanism(s) that are occurring in the inhibition process. Hitherto there has been some very general speculation[1] about the mechanism of the inhibition reactions. However, no mechanistically orientated studies that involve amino acids or even amines reacting with sulfamate esters or sulfamoyl chlorides (precursors for *N*-sulfonylamines such as $[HN{=}SO_2]$ or $[RN{=}SO_2]$) have been reported. Thus our recent results and those of Thea and Williams[21] are the only ones that throw light on the reaction mechanism(s) taking place.

Present and future studies include exploration of structure-reactivity relationships on the β_1 and β_2 segments of the plot, deuteration studies and work on 667-Coumate and Emate and possibly other biologically active sulfamate esters.

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