Tetrahedron Letters 51 (2010) 2059–2062

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Mechanism of the hydrolysis of the sulfamate EMATE—an irreversible steroid sulfatase inhibitor

William J. Spillane *, Jean-Baptiste Malaubier

School of Chemistry, National University of Ireland, Galway, Ireland

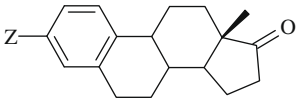
ARTICLE INFO

Article history:
Received 3 December 2009
Revised 4 February 2010
Accepted 12 February 2010
Available online 16 February 2010

ABSTRACT

The kinetics of hydrolysis of the medicinally important sulfamate ester EMATE have been probed over a wide pH range and into moderately strong base (H_ region). Analysis of the pH/H_-rate profile, measurements of pK_as, solvent-reactivity, kinetic isotope effects and determination of activation data reveal that in the pH range from ~1 to ~8 an S_N2 (*S*) solvolytic mechanism is followed and after the pK_a of EMATE (pK_a ~9) is passed, a second pathway showing a first-order dependence on base operates and an E1cB mechanism is supported.

The importance of certain types of sulfamate esters in medicinal chemistry has been outlined[1,2] and numerous authoritative reviews have appeared in this area.[3–6] However the mechanism(s) of action of these medicinally-important esters while speculated on in the literature[7–9] remains uncertain, though very recent work[10,11] has thrown new light on the mechanism(s) through which they inactivate arylsulfatases. In earlier work the hydrolysis and aminolysis of simpler sulfamate esters[12,13] have been examined thoroughly in aqueous media and in acetonitrile and the principal reaction pathways have been eliminative in character with E1cB and E2 mechanisms being supported. However, recently a non-eliminative mechanism[1] has been found under conditions (pH ~2.7) that do not allow the removal of a hydrogen from the ester, NH_2SO_2O–.



1a: Z = NH_2SO_2O
1b: Z = $^-NHSO_2O$
1c: Z = $^{2-}NSO_2O$
2a: Z = HO
2b: Z = ^-O

The purpose of this Letter is to explore, especially under conditions that are as close as possible to those employed in clinical application, the physical organic chemistry of an important biologically active sulfamate ester, namely, estrone 3-*O*-sulfamate (EMATE) **(1a)**. EMATE was the first active site directed irreversible sulfatase inhibitor to be discovered. It was chosen for this study because it represents a good generic example from the field of medicinally relevant sulfamate esters. A sample of EMATE was readily available (see Acknowledgments) and a sample was subsequently synthesized in this laboratory by standard procedures.[14]

An array of techniques has been used viz., (pH/H_)-rate profile, solvent-reactivity studies (Grunwald–Winstein, extended Grunwald–Winstein and nucleophilicity tests), kinetic isotope effects and measurement of thermodynamic data. Some data have also been collected under non-biological conditions, for example, at alkaline pHs and the lower H_ region in order to probe the mechanism over the whole range of the pH/H_-rate profile.

The (pH/H_)-rate profile data (see Supplementary data) for **1a** is plotted in Figure 1. Rates were measured in water at 50 °C over the pH range 1.74–13.69 and in strong alkali up to 3 M KOH (H_ region). The rates of the formation of estrone **2a** (at λ = 275 nm), or under more alkaline conditions, of anionic estrone **2b** (at λ = 296 nm) were followed. Rates did not vary more than 5% based on the average of three runs. Constant ionic strength (μ = 1.0) was

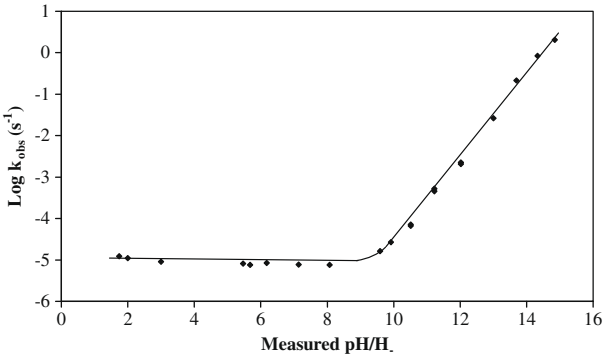


Figure 1. (pH/H_)-rate profile data at 50 °C for **1a**.

* Corresponding author. Tel.: +353 91 492475; fax: +353 91 525700.
E-mail address: william.spillane@nuigalway.ie (W.J. Spillane).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.02.065

maintained with KCl over the pH range studied and solutions were buffered with a stock solution of 0.01 M TRIS-cacodylate. In some cases at pHs ~11–12 the increase in both **2a** and **2b** could be followed simultaneously and the variation in rate was at maximum 7%. Product studies carried out at several points on the pH/H_ profile showed that **2a/2b** formed almost quantitatively. For example, the absorbance of a 'spent' kinetic solution of **1a** in 1.5 M KOH was within 4% of the absorbance measured for a freshly made-up 1×10^{-4} M solution of **2b**. The agreement between spent and spiked solutions was always within 6%.

The first pK_a of **1a** (loss of the first proton from NH_2SO_2O–) can be derived from the pH/H_-rate profile below as 9.45. This kinetic pK_a is in good agreement with the UV value of 9.5 in 70% aqueous MeOH.[15] In water, the second pK_a has not been determined but based on these pK_a values and spectrophotometric determinations in this laboratory in MeCN, which gave pK_a values of 15.1 and 21.4 for the first and second ionizations, respectively, it should be ~15–16.[16] The hydrogen α to the carbonyl group in **1a** will have a pK_a in water of ~16.5 since this is the reported value for cyclopentanone.[17] This pK_a should be virtually the same for both **1b** and **1c** since the distance from the nitrogen centre to the carbonyl centre is considerable and any effect on the ionization would be minimal.

The pH/H_-rate profile displays two distinct regions reminiscent of those obtained for the simpler sulfamate $NH_2SO_2OC_6H_4NO_2$–*p*.[12] However, the change in the slope from zero to unity for this ester occurs at about pH 5 whereas for **1a** this change occurs at about pH 9.5. This lateral shift is due to the fact that the pK_a of the former is 7.29 while the corresponding pK_a for **1a** is about 2 pK_a units higher. In the pH-independent region the reacting species will be **1a**, and above the pK_a of **1a** ~9.5, it will be **1b** and eventually **1c**. Two important conclusions can be made from examination of Figure 1. First the upward curvature indicates that a change in mechanism occurs,[18,19] and second, in the pH-independent region there is no catalysis and the upward line has a slope of 1 indicating that the process occurring is first order in [OH^-].

In order to probe the mechanism on the non-catalyzed section of the pH/H_ profile a Grunwald–Winstein plot (see Supplementary data) was constructed using twelve rate constants at 50 °C for reaction of **1a** in aqueous EtOH mixtures containing from 90% to 20 water content and using Y_{OTs} values[20] for these solutions (Table 1). The pH ranged from 5.68 (90% water content) to 6.45 (20% water content).

The plot yields an *m* value of 0.13 (correlation coefficient, *r* = 0.97). Values of *m* at this level are interpreted as indicating support for a bimolecular mechanism; in this case, most likely an S_N2 mechanism involving attack by water at the sulfur of **1a**. The data can be scrutinized in another way using the extended Grunwald–Winstein equation (see Supplementary data),[21]

Table 1
Rate data for hydrolysis of **1a** in aqueous EtOH and parameters for Grunwald–Winstein plots

Water–Ethanol	Y_{OTs}	N_{OTs}[b]	Log (k_{obs})[c] (s^{-1})
90–10	3.78	−0.41	−4.73
80–20	3.32	−0.34	−4.78
75–25	3.11[a]	–	−4.86
70–30	2.84	−0.35	−4.95
60–40	1.97	−0.23	−5.04
56.8–43.2	1.92	—	−5.06
55.2–44.8	1.83	—	−5.07
50–50	1.29	−0.09	−5.09
40–60	0.92	−0.08	−5.12
30–70	0.47	−0.05	−5.20
25–75	0.23[a]	—	−5.22
20–80	0.00	0.00	−5.24

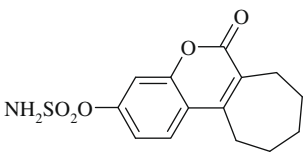
[a] These two Y_{OTs} values were interpolated from the literature.[20]
[b] N_{OTs} values were taken from the literature.[20]
[c] The rates are the average of three runs with less than 4% deviation.

$$\text{Log } k_{obs} = mY_{OTs} + lN_{OTs}$$

where the second term measures the sensitivity (*l*) of the hydrolysis to nucleophilicity through the use of the N_{OTs} parameter which is a measure of the nucleophilicity of each solvent mixture. *A priori* since *m* is low, one would expect for this hydrolysis that the nucleophilicity would be important and this is the case; an *l* value of 0.93 and an *m* value of 0.24 being found from the plot for the extended equation (*r* = 0.98). These values strongly support a mechanism involving nucleophilic attack by water in the hydrolysis of **1a** and a lesser role for the ionizing power of the solvent. In the extended equation the *m* value is expected to be somewhat different to that found in the basic Grunwald–Winstein equation. Kevill has suggested that *l*/*m* ratios of greater than 1.6 indicate a bimolecular mechanism and values between 0 and 1 may be found for unimolecular reactions.[22,23] In the present study the *l*/*m* value is 3.9. Hydrolysis studies by the Kevill group on series of carbonyl-[22] and sulfonyl-[23] chlorides showed that *l*/*m* values falling into both categories were found for the former and they were assigned to 'ionization' or addition–elimination mechanisms, respectively, whereas for the latter all the values were ~2 and they were interpreted as indicating an S_N2 solvolytic displacement mechanism. For an *N*-acylsulfamate ester of the type, R-$CONHSO_2O$-Ar, which was found to undergo an S_N2 type reaction, an *l*/*m* value of 3.3 may be calculated from data for its hydrolysis in similar aqueous EtOH mixtures.[24]

Another type of solvent-reactivity mechanistic probe that can be applied here involves the use of two solvent mixtures with identical ionizing power (same Y_{OTs}) but very different nucleophilicities (differing N_{OTs}). If the hydrolysis is significantly slowed down in the solvent of lower nucleophilicity, this suggests that the solvent is playing an important role as a nucleophile. The results of the application of this method to the hydrolysis of **1a** are given in Table 2. It can be readily seen that employing 97% trifluoroethanol (TFE), 85% TFE and 97% hexafluoro-*i*-propanol (HFIP) results in a moderate reduction in rate thus pointing to an important role for water as a nucleophile in the hydrolysis of **1a**. Curiously, though the ratios in Table 2 are considerably lower than those observed for the hydrolysis of other compounds including the simpler ester $NH_2SO_2OC_6H_4NO_2$-*p*.[1] The hydrolysis of the ester 667-COUMATE (**3**), which is the subject of considerable interest, gives ratios in line with those in Table 2.[25]

Kinetic solvent isotope effects (KSIEs) k_{H2O}/k_{D2O} for **1a** of 3.93 (pD = 6.61, at 60 °C) and 5.48 (pD = 12.90, at 15 °C) have been determined. The former value supports a bimolecular S_N2 pathway[1] in the flat region of the pH/H_ rate profile. The reacting species will be *d*$_2$-**1a** because of rapid exchange of the protons for deuterons in D_2O however, this will not contribute to the isotope effect since the ND_2 centre is not involved in the slow step of the S_N2 reaction at sulfur and the value of 3.93 is a KSIE. At pD 12.90 on the ascending part of the profile *d*-**1b** will be deuterated on nitrogen and the isotope effect observed (5.48) is a KIE effect reflecting extensive cleavage of the deuterium-nitrogen bond in the slow step of an E1cB reaction.[26]



3

Activation data were determined for the hydrolysis of **1a** at various pHs and in 1.5 M and 3 M KOH. The energy of activation E_a was obtained from the Arrhenius equation and the heat of activation $\Delta H^{\ddagger}$ and the change in entropy $\Delta S^{\ddagger}$ were calculated from the Eyring equation. Table 3 tabulates the values obtained and the

Table 2
Nucleophilicity data for the hydrolysis of **1a** at 50 °C

Solv. mixture (w/w)	N_{OTs}	Y_{OTs}	$k_{obs} \times 10^6$ (s^{-1})	Rate ratio $k_{solv\ mixture}/k_{solv.\ low\ nucl.}$
44.8% EtOH	−0.19	1.83	8.51	6.03[a]
52.4% MeOH	−0.17	1.83	6.51	4.61[a]
36.0% MeCN	−1.16	1.83	5.89	4.17[a]
43.2% EtOH	−0.20	1.92	8.71	4.58[b]
50.5% MeOH	−0.18	1.92	8.56	4.50[b]
34.5% MeCN	−1.16	1.92	4.65	2.44[b]
10% EtOH	−0.41	3.78	18.8	2.01[c]
10% MeOH	−0.41	3.78	16.4	1.74[c]
10% MeCN	−1.29	3.60	16.1	1.71[c]

[a] Rate ratio against hydrolysis of **1a** in 97% TFE ($N_{OTs} = -3.25$, $Y_{OTs} = 1.83$), $k_{obs} = 1.41 \times 10^{-6}\ s^{-1}$.
[b] Rate ratio against hydrolysis of **1a** in 85% TFE ($N_{OTs} = -2.01$, $Y_{OTs} = 1.92$), $k_{obs} = 1.90 \times 10^{-6}\ s^{-1}$.
[c] Rate ratio against hydrolysis of **1a** in 97% HFIP ($N_{OTs} = -4.27$, $Y_{OTs} = 3.60$), $k_{obs} = 9.4 \times 10^{-6}\ s^{-1}$.

Table 3
Activation data for the hydrolysis of **1a**

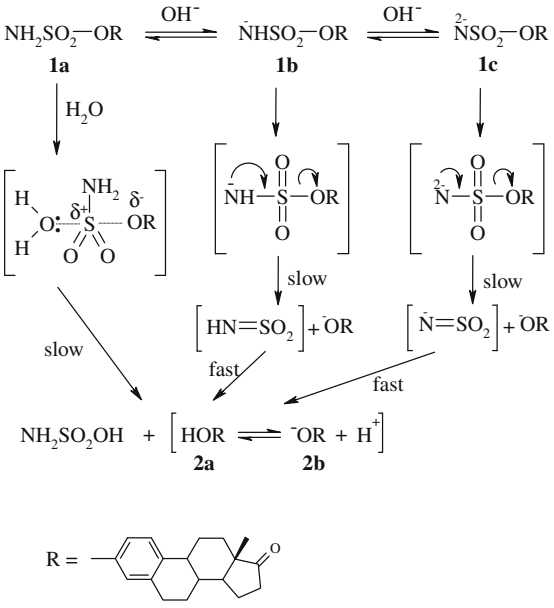
pH/H_	Temp. range[a] (K)	E_a (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})
1.74	333–358	95	92	−52
9.59	313–353	88	86	−69
13.69	278–318	78	76	−25
14.33[b]	283–308	76	74	−19
14.85[c]	278–298	75	73	−15

[a] Rates were measured at 5 K intervals over the range. The correlation coefficients (r) of the Arrhenius and Eyring plots were ⩾0.98.
[b] This H_ corresponds to 1.5 M KOH.
[c] This H_ corresponds to 3 M KOH.

footnotes, the number of points used and the range of temperatures for each determination. The entropies at pHs 1.74 and 9.59 are reasonably negative and would support a bimolecular mechanism involving exclusively **1a** (at pH 1.74) and **1a/1b** (at pH 9.59). At pH 13.69 and in stronger base **1b** and **1c** will be the species present and since the first step of an elimination path (removal of a proton) has been realized the mechanism here is likely to be E1cB. The negative entropies measured at pHs 1.74 and 9.59 support an S_N2 mechanism and the less negative entropies suggest a unimolecular slow decomposition of **1b** and **1c**.

In Scheme 1 the slow S_N2 solvolytic pathway for the decomposition of **1a** in the acidic pH region is shown on the left, in the centre an E1cB mechanism involving the hydrolysis of monoanionic **1b** and on the right a similar mechanism involving dianionic **1c** is shown. Various *N*-sulfonylamines are envisaged in the eliminative routes (Scheme 1) as previously proposed for the hydrolysis of $NH_2SO_2OC_6H_4X$.[12] All routes give the same final products, namely, sulfamic acid and estrone, as either **2a** or **2b**.

Recently the kinetics of inactivation of an arylsulfatase (from *Pseudomonas aeruginosa*) by simple sulfamates $NH_2SO_2OC_6H_4X$ have shown that there is a high degree of cleavage of the XC_6H_4O-S bond ($\beta_{lg} = -1.1$)[10] in inactivation and this Brønsted value is in close agreement with the value of -1.2[12] reported for the hydrolysis of similar sulfamates at pH 7 where an E1cB mechanism was favoured. For hydrolysis at pH 2 the same sulfamates gave a much smaller β_{lg} of −0.41[1] and this, in conjunction with other findings, supported an S_N2 mechanism where cleavage of the O–S bond would be expected to be less advanced in the transition state. In the current work with **1a** however, it is important to note that at pH ~9 and below this value an S_N2 (S) mechanism at the sulfur atom occurs. At higher pH an E1cB mechanism will occur. This arises because the pH/H_-rate profile for **1a** differs from that for the simpler esters, being as pointed out above, laterally shifted



Scheme 1. Proposed mechanisms of hydrolysis of **1a**, **1b** and **1c**.

by about two units. The incursion of a path involving the dianionic sulfamate, **1c**, arises in the strongest base as the second pK_a of **1a**, that is, **1b** ⇆ **1c** is approached.[2]

A range of physical organic mechanistic techniques have indicated that the hydrolysis of EMATE (**1a**) occurs by two different mechanisms: an S_N2 (S) mechanism below pH ~9.5 and E1cB mechanisms involving *N*-sulfonylamines at higher pHs.

Acknowledgements

Professor B. V. L. Potter, Department of Pharmacy and Pharmacology, University of Bath and Sterix Ltd. is thanked for kindly providing a gift of EMATE. Professor Potter is also thanked for many helpful suggestions during the preparation of this Letter. Professor S. Thea, University of Genova, Italy is thanked for advice regarding pK_a values. The NUI, Galway Millennium Fund is thanked for financial support.

Supplementary data

Supplementary data (a table containing kinetic data for the pH/H_-rate profile for **1a** at 50 °C, a figure showing the Grunwald–Winstein plot for **1a** in aqueous EtOH at 50 °C and a figure showing the extended Grunwald–Winstein plot for **1a**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.065.

References and notes

1. Spillane, W. J.; McCaw, C. J. A.; Maguire, N. P. *Tetrahedron Lett.* **2008**, *49*, 1049–1052.
2. Spillane, W. J.; O'Byrne, A.; McCaw, C. J. A. *Eur. J. Org. Chem.* **2008**, 4200–4205.
3. Winum, J.-Y.; Scozzafava, A.; Montero, J.-L.; Supuran, C. T. *Expert Opin. Ther. Patents* **2004**, *14*, 1273–1308.
4. Winum, J.-Y.; Scozzafava, A.; Montero, J.-L.; Supuran, C. T. *Med. Res. Rev.* **2005**, *25*, 186–228.
5. Reed, M. J.; Purohit, A.; Woo, L. W. L.; Newman, S. P.; Potter, B. V. L. *Endocr. Rev.* **2005**, *26*, 171–202.
6. Foster, P. A.; Reed, M. J.; Purohit, A. *Anticancer Agents Med. Chem.* **2008**, *8*, 732–738.
7. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508–11514.
8. Hanson, S. R.; Best, M. D.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2004**, *43*, 5736–5763.

9. Woo, L. W. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. L. *Chem. Biol.* **2000**, *7*, 773–791.
10. Bojarová, P.; Denehy, E.; Walker, I.; Loft, K.; De Souza, D. P.; Woo, L. W. L.; Potter, B. V. L.; McConville, M. J.; Williams, S. J. *ChemBioChem* **2008**, *9*, 613–623.
11. Bojarová, P.; Williams, S. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 477–480.
12. Thea, S.; Cevasco, G.; Guanti, G.; Williams, A. *J. Chem. Soc., Chem. Commun.* **1986**, 1582–1583.
13. Spillane, W. J.; McGrath, P.; Brack, C.; O'Byrne, A. B. *J. Org. Chem.* **2001**, *66*, 6313–6316.
14. Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068–1083.
15. Sahm, U. G.; Williams, G. J.; Purohit, A.; Hidalgo Aragones, M. I.; Parish, D.; Reed, M. J.; Potter, B. V. L.; Pouton, C. W. *Pharm. Sci.* **1996**, *2*, 17–20.
16. Thea, S., Private Communication.
17. Hennig, L.; Alva-Astudillo, M.; Mann, G. K. T. *Monatsh. Chem.* **1992**, *123*, 571–579.
18. Loudon, G. M. *J. Chem. Educ.* **1991**, *68*, 973–984.
19. Page, M.; Williams, A. *Organic and Bio-organic Mechanisms*; Addison Wesley Longman: Harlow, UK, 1997.
20. Bentley, T. W.; Llewellyn, G. Y_X *Scales of Solvent Ionizing Power*, 1990.
21. Kevill, D. N.; D'Souza, M. J. *J. Chem. Res.* **2008**, 61–66.
22. Kevill, D. N.; Kyong, J. B.; Won, H. *Int. J. Mol. Sci.* **2005**, *6*, 87–96.
23. D'Souza, M. J.; Yaakoubd, L.; Mlynarski, S. L.; Kevill, D. N. *Int. J. Mol. Sci.* **2008**, *9*, 914–925.
24. Spillane, W. J.; Malaubier, J.-B. *Tetrahedron Lett.* **2007**, *48*, 7574–7577.
25. Maguire, N. P. Ph.D. Thesis, National University of Ireland, 2009.
26. Cockerill, A. F.; Harrison, R. G. In *Chemistry Double-Bonded Functional Groups*; Wiley: Chichester, 1977; pp 149–329.