Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information for

Kinetic and computational evidence for an intermediate in the hydrolysis of sulfonate esters

Ann C. Babtie,[1] Marcelo F. Lima,[1,2,3] Anthony J. Kirby[2]* & Florian Hollfelder[1]*

[1]Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA.

[2]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

[3] Present address: LQBOA – Campus Universitário do Araguaia, Universidade Federal de Mato Grosso, Barra do Garças-MT, ZIP code 78600-000 – Brazil

*Correspondence: E-mail: ajk1@cam.ac.uk, fh111@mole.bio.cam.ac.uk; Fax: (+)44-1223-766002.

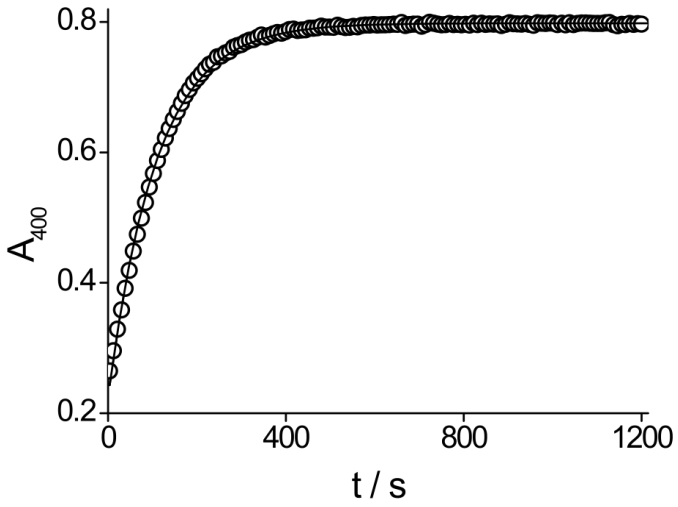


Figure S1. *Kinetics of the reaction of 4-nitrophenyl benzenesulfonate* **1**. (A) A typical progress curve ([4-nitrophenyl benzenesulfonate] = 0.04 mM, T = 50°C, 0.1 M KOH). Pseudo-first order rate constants k_{obs} were obtained from exponential fits of progress curves (using $A_t = A_\infty - (A_\infty - A_0)e^{-kt}$)

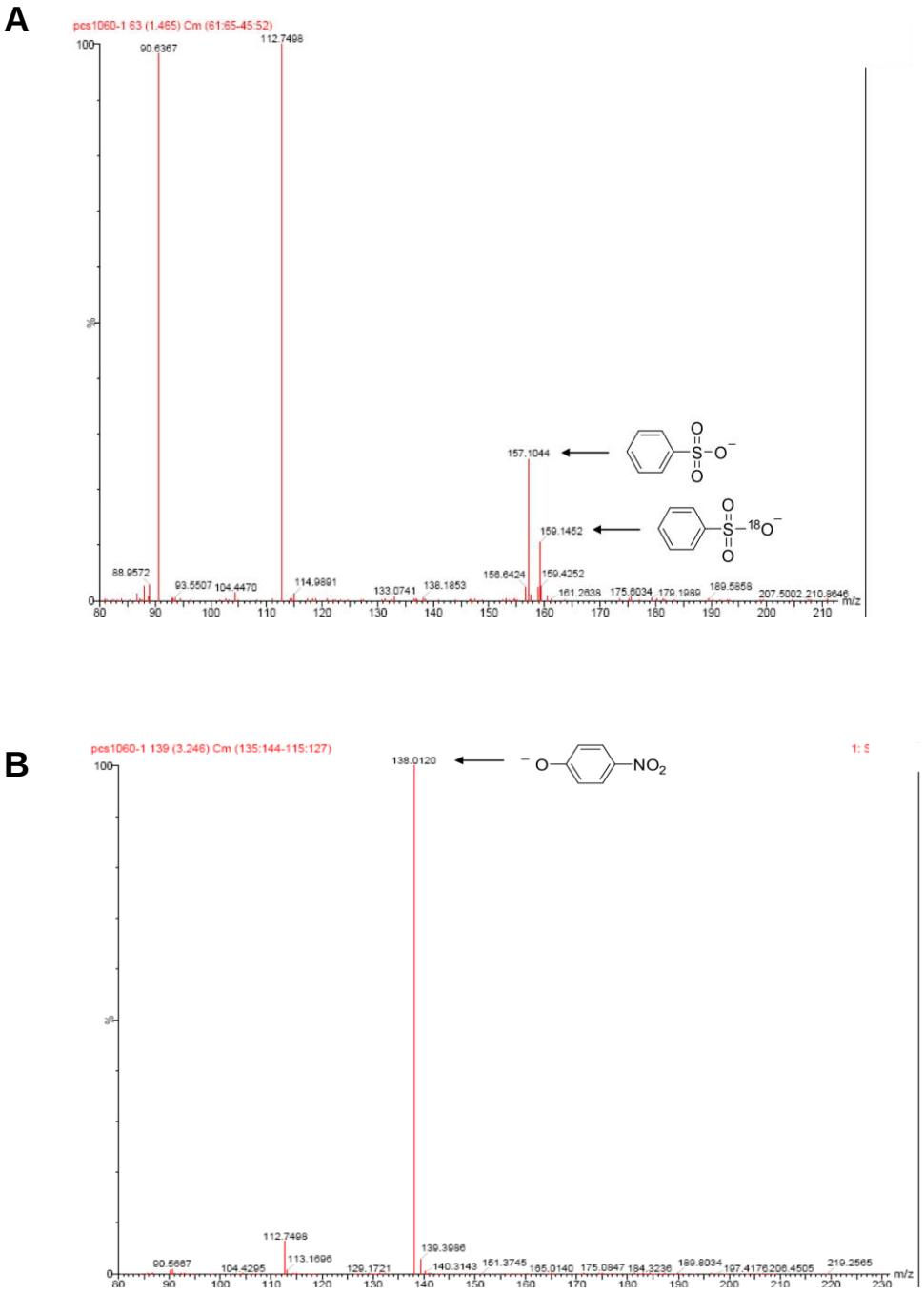
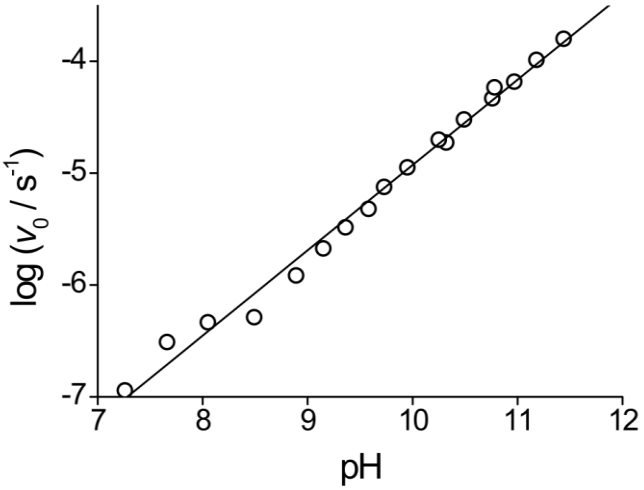


Figure S2: LC-MS of products of 4-nitrophenyl benzene sulfonate hydrolysis in the presence of 38 % $H_2{}^{18}O$. ^{18}O was only detected in the $PhSO_3^-$ product (A); the $(O_2N)C_6H_4O^-$ product was unlabelled (B).

A



B

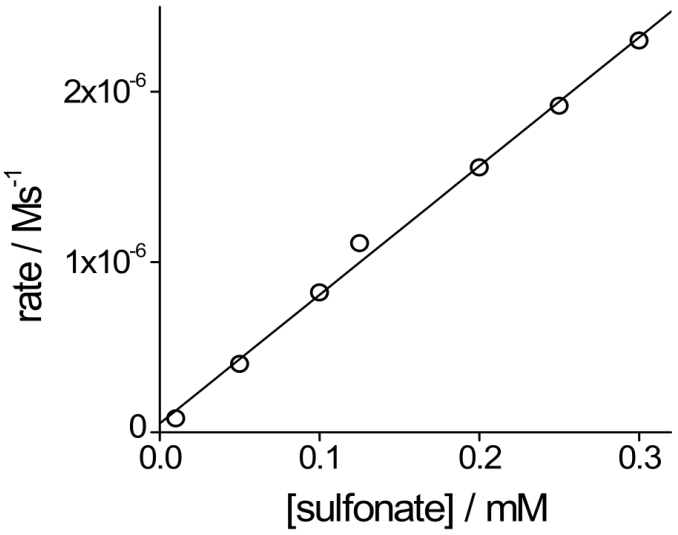


Figure S3. *Kinetic plots for the hydrolysis of 4-nitrophenyl benzenesulfonate* **1**. (**A**) The pH-rate profile of the hydrolysis of 1 is linear (r = 0.99), establishing first order kinetics with respect to the hydroxide nucleophile. *Conditions:* 0.01-0.12 M KOH; T = 50°C; 0.25 mM sulfonate (**B**) The initial rate correlates linearly to sulfonate ester concentration. *Conditions*: 0.1 M KOH; T = 50 °C; 0.01-0.3 mM sulfonate.

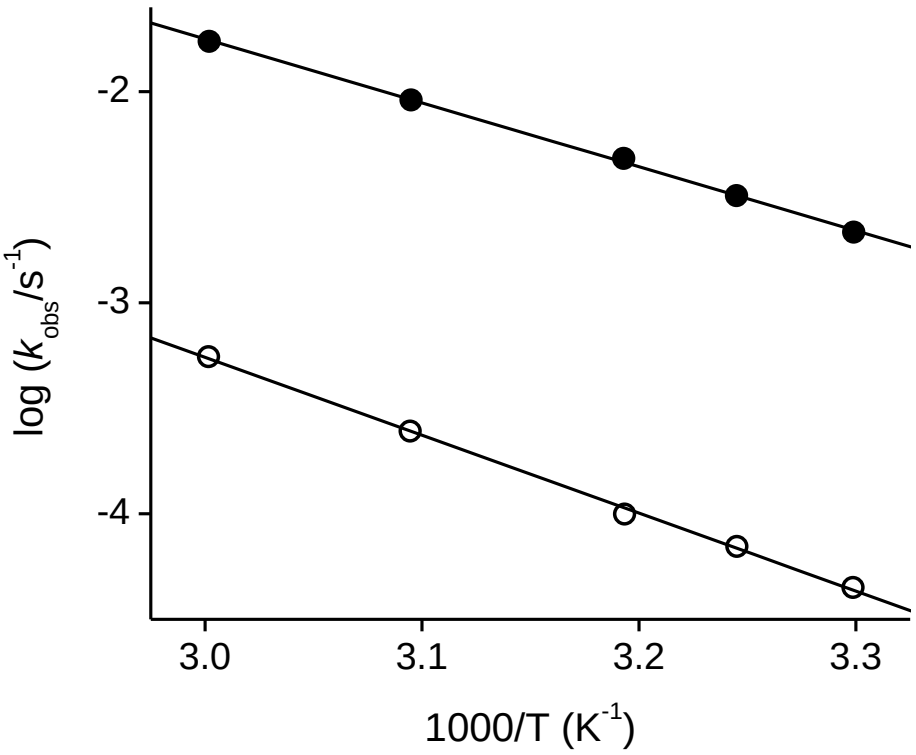


Figure S4: Arrhenius plots for the alkaline hydrolysis of phenyl- and *p*-nitrophenyl benzenesulfonates. Phenyl benzenesulfonate (open circles; gradient = -3.7±0.1, y-intercept = 7.8±0.3), 4-nitrophenyl benzenesulfonate (filled circles; gradient = -3.0±0.1, y-intercept = 7.3±0.2). *Conditions:* 0.1 M KOH; T = 30-60 °C.

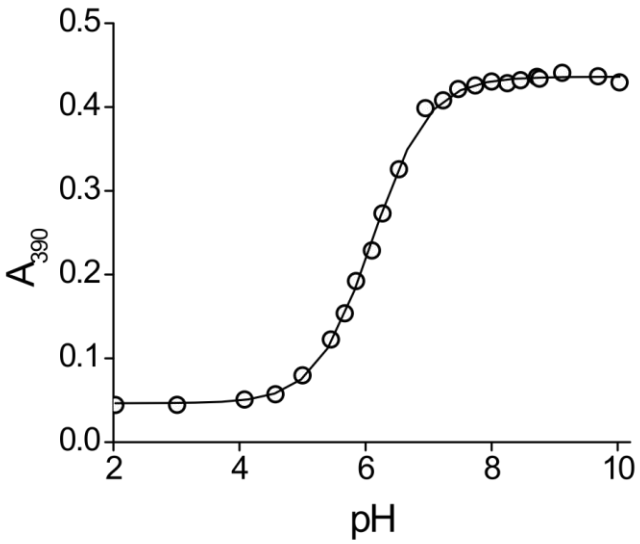


Figure S5: Determining pK_a of 3-fluoro-4-nitrophenol. *Conditions*: 100 mM buffers (pH adjusted with HCl or KOH): CHES (pH 9-10), Tris (pH 7-9), MES (pH 5.5-6.5), acetic acid (pH 4-5), phosphoric acid (pH 2-3); [3-fluoro-4-nitrophenol] = 30 μM; T = 25 °C; 200 μl total volume in 96-well plates (Nunc). A pK_a value of 6.11 ± 0.01 was determined by fitting to the following equation: $A_{390} = A_2 + (A_1-A_2)/(1+\exp((pH-pK_a)/dx))$ where A_1 and A_2 are the lower and upper bounds of A_{390} respectively and dx is the slope factor.

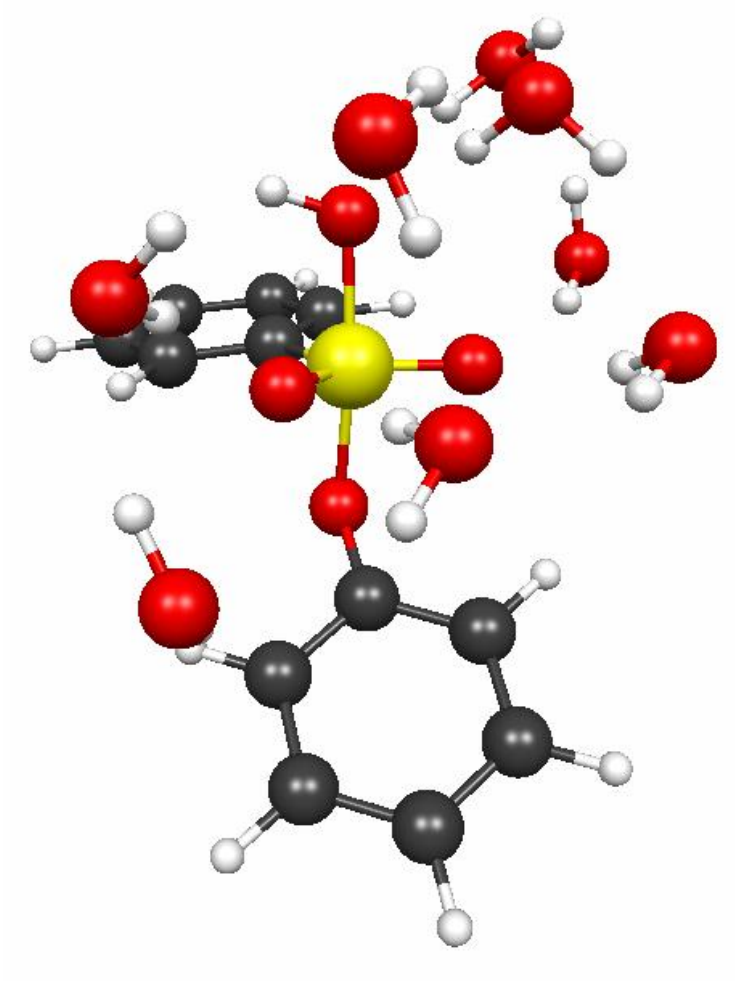


Figure S6. Coloured ball and stick model of the calculated structure shown in Figure 2.

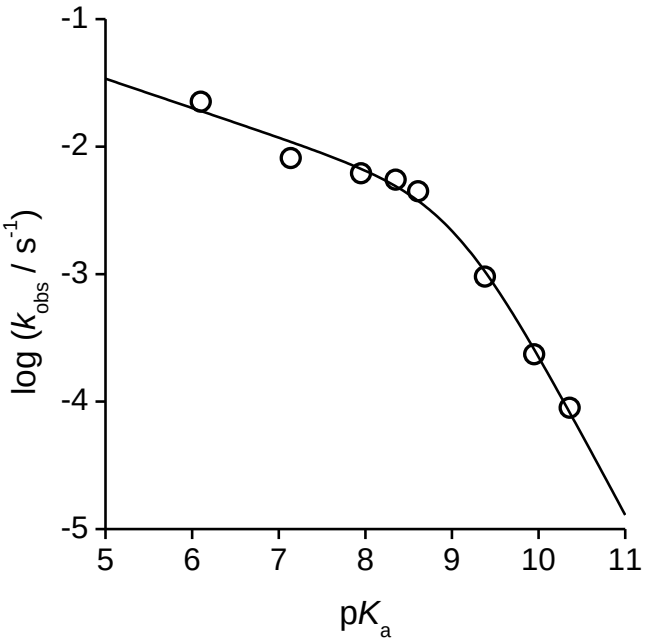


Figure S7: A fit of the data shown in Figure 1 of the main text to a composite of two curves with different slopes (corresponding to a shifting TS structure in an alternative concerted mechanism). Fitting to the equation log k_{obs} = A_1 + (β_{LG}^{left}*pK_a) – log (1 + 10^((β_{LG}^{left}-β_{LG}^{right})*(pK_a-A_2))), yielded the following parameters: β_{LG}^{left}= -0.23 ± 0.07; β_{LG}^{right}= -1.3 ± 0.2 ; A_1= - 0.3 ± 0.5 (intercept at pK_a zero); A_2 = 9.1 ± 0.3 (breakpoint).

Table S1. Parameters for the substituted phenyl benzenesulfonates tested in this work. The substituent on the leaving group phenol (X in Scheme 1) is used to identify the sulfonate. The leaving group pK_a values (at T= 25 °C) and mean log(k_{obs}) values used in Figure 1 are listed. The wavelengths used to follow accumulation of the hydrolysis product of each sulfonate ester are given. *Conditions:* 0.1 M KOH; T = 50 °C.

X	pK_a	λ / nm	log(k_{obs}/s^{-1})
3-F-4-NO_2	6.11	440	-1.65
4-NO_2	7.14	440	-2.09
4-CN	7.95	276	-2.21
3-NO_2	8.35	395	-2.26
3-CN	8.61	291	-2.35
4-Cl	9.38	299	-3.02
H	9.95	280	-3.63
3,4-diMe	10.36	237	-4.05

Table S2. Cartesian coordinates of the calculated intermediate structure shown in Figures 2 and S6.

S	-0.1409	-0.1782	-0.4444
O	-0.2728	-0.2127	1.0083
O	1.1345	-0.0858	-1.1428
C	-1.6346	-0.3664	-1.4112
O	-0.1387	-2.0286	-0.5507
O	-0.3653	1.5550	-0.5442
C	-1.5723	-0.2537	-2.7881
C	-2.7453	-0.3594	-3.5224
C	-3.9576	-0.5715	-2.8797
C	-3.9985	-0.6815	-1.4965
C	-2.8300	-0.5885	-0.7526
H	-0.6354	-0.0823	-3.2827
H	-2.7086	-0.2758	-4.5937
H	-4.8639	-0.6493	-3.4529
H	-4.9343	-0.8506	-0.9948
H	-2.8548	-0.6945	0.3158
C	0.5915	-2.7787	0.2880
H	-1.1315	1.8131	-0.0410
C	-0.0500	-3.5186	1.2845
C	0.6751	-4.3524	2.1235
C	2.0568	-4.4545	1.9961
C	2.6959	-3.7222	1.0052
C	1.9744	-2.8944	0.1542
H	-1.1175	-3.4359	1.3837
H	0.1608	-4.9292	2.8739
H	2.6191	-5.0980	2.6492
H	3.7635	-3.7957	0.8882
H	2.4741	-2.3335	-0.6127
O	1.2867	2.5088	3.5220
H	1.8131	1.7356	3.3958
H	1.2824	2.9713	2.6992
O	1.7024	3.2063	0.7039
H	2.5163	2.7463	0.5742
H	1.0327	2.6992	0.2735

O	-0.9348	0.5444	3.8009
H	-0.3686	1.2966	3.8676
H	-0.9906	0.3274	2.8840
O	3.5832	0.9668	0.2338
H	3.5127	0.5763	1.0902
H	2.9128	0.5717	-0.3004
O	2.2695	-0.0523	2.6437
H	2.2559	-0.7111	3.3195
H	1.4992	-0.1891	2.1158
O	0.3102	3.0681	-2.9558
H	0.7113	3.8753	-2.6757
H	0.0363	2.6157	-2.1740
O	2.0771	0.9015	-3.8127
H	1.5886	1.7075	-3.7616
H	1.9177	0.4362	-3.0071
O	0.7503	-1.7799	4.5125
H	0.1182	-1.0828	4.4393
H	0.4887	-2.4487	3.8999