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Accurate Kinetic Studies by High-Performance Liquid Chromatography

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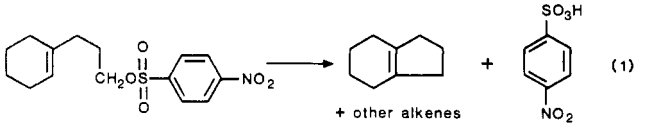
High-performance liquid chromatography (HPLC) is applied routinely in organic chemistry to quantitative analyses of products and to semiquantitative studies of reactions in progress.[2] We now report that good quality rate constants can be obtained with standard equipment by direct analysis of microaliquots (<2 μL) and without addition of an internal standard.

We have studied rates and products of the cyclization of 3-(cyclohex-1-enyl)propyl *p*-nitrobenzenesulfonate (I) in hexafluoroisopropyl alcohol (HFIP) containing added buffers and salts. In anticipation of a requirement for kinetic studies in the presence of an excess of substituted pyridines as buffers, we have developed an alternative to the usual spectrophotometric method.[3] A direct method based on HPLC proved to be surprisingly accurate. As reversed-phase HPLC is a very versatile and reproducible analytical method,[2] it seems likely that there will be many other suitable applications.

Results

Peak areas for both appearance of the sulfonic acid (eq 1) and disappearance of the ester I were obtained, after



(1)

I

direct injection of quenched aliquots of *only 1.8 μL* of the HFIP solution into the HPLC loop-injection valve. Rate constants for these two almost independent measurements agree well (Table I)—the average discrepancy between appropriate pairs of values is only 2.4%. More detailed inspection showed, as might have been expected, that a primary source of experimental uncertainty was slight variation (often <1%) in the volume of solution injected. Correction of these small errors was based on the linear relationship between peak areas for acid and ester (Table II). Our best estimates of the rate constants are the normalized values (Table I), and a brief, general explanation of the normalization procedure will now be given.

The symbols S and P will refer to observed peak areas (integrator counts) of the *s*tarting material and *p*roduct, respectively. Also the peak areas corresponding to the initial concentration of starting material and the final concentration of product are S_0 and P_∞, respectively. When a constant volume is injected and the combined concentrations of starting material and product are unchanged, then eq 2 applies (see also Table II). Corrected

$$P + (P_\infty/S_0)S = P_\infty \quad (2)$$

values of S and P, referred to as S' and P', can be obtained from eq 2 (with $P = P'$, $S = S'$, and the appropriate P_∞ and S_0 values given in Table II) and eq 3, which assumes that the ratio of peak areas (P/S) is accurate.

$$P/S = P'/S' \quad (3)$$

From these two equations it can be shown that each pair of values of P and S needs to be multiplied by an individually calculated scaling factor, given by $P_\infty/\{P + (P_\infty/S_0)S\}$, to obtain the corresponding corrected values P' and S'. In practice only one set of calculations is required (correcting independently each value of either P or S), because both sets of values (P' or S') lead to the same value of the rate constant (see the normalised values in the final column in Table I).[5]

Discussion

The normalized values of the rate constants (Table I) are close to, but different from, the averages of the corresponding rate constants obtained from acid and ester peaks. The statistical uncertainty in the normalized values varies from only ±1.3 to ±2.4% of k. The high reproducibility of the chromatography is shown by the very good agreement between the relative response ratios P_∞/S_0 (Table II), even for wide variations in the acidity or basicity of the solutions and the nature of added buffers or salts. Only for run 2 is the value of P_∞/S_0 significantly higher than the other values, and the normalized rate constant quoted for run 2 (Table I) probably includes a small systematic error decreasing the calculated rate constant by 3–5%. This conclusion is based on trial calculations of the effect of incorrect values of P_∞/S_0[6] and on a plot of rate constants vs molar concentration of triethylamine (runs 1–5). An independent kinetic run, duplicating closely the conditions of run 2 but 5 months later, gave the expected value of P_∞/S_0 (2.76 ± 0.07) and a significantly higher rate constant ((1.65 ± 0.05) × 10^{-4} s^{-1}, based on 20 HPLC injections). Thus, from the slightly high value of P_∞/S_0 for the original run 2, it can be correctly predicted that the rate constant is slightly too low.

As well as providing a good guide to the reliability of the data, the plots of P vs. S (eq 2, Table II) provide a means in future work to establish a reliable rate constant from relatively few (10–15) HPLC injections for each kinetic run. For most of the work reported here, we made about 40

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(5) It can be shown that the integrated form of the first-order rate equation is the same for calculations of rate constants determined from the increase in P' or the decrease in S'.
(6) For run 6, normalised values of 10^4k calculated from different values of P_∞/S_0 are as follows: 2.326 (Table I), 2.711 (Table II), 2.277, 2.811; 2.237, 2.911.

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Table I. Rate Constants for Solvolyses of 0.01 M 3-(Cyclohex-1-enyl)propyl *p*-Nitrobenzenesulfonate (I) in Hexafluoroisopropyl Alcohol at 100.0 °C

		method of calculation[a]		
run	buffer or salt	acid peak[b] $10^4k/s^{-1}$	ester peak[c] $10^4k/s^{-1}$	normalised[d] $10^4k/s^{-1}$
1	none present[e]	1.398 ± 0.010	1.472 ± 0.030	1.445 ± 0.019
2	NEt_3 (0.02 M)[f]	1.462 ± 0.035	1.528 ± 0.032	1.496 ± 0.028
3	none present[e]	1.466 ± 0.048[g]	1.442 ± 0.033	1.446 ± 0.030
4	NEt_3 (0.05 M)	1.710 ± 0.070	1.748 ± 0.063	1.755 ± 0.043
5	NEt_3 (0.08 M)	1.933 ± 0.040[h]	1.979 ± 0.037[h]	1.967 ± 0.029[h]
6	2,6-DTBP (0.08 M)[i]	2.250 ± 0.055	2.332 ± 0.068	2.326 ± 0.036
7	$NEt_4^+BF_4^-$ (0.08 M)	1.631 ± 0.037	1.629 ± 0.063	1.639 ± 0.039
8	urea (0.08 M)	1.516 ± 0.038	1.518 ± 0.040	1.519 ± 0.031

[a] Errors shown are standard deviations. [b] Rate constant obtained by monitoring directly the appearance of *p*-nitrobenzenesulfonic acid. [c] Rate constant obtained by monitoring directly the disappearance of the nosylate ester. [d] Rate constant obtained from scaled values of the peak areas for acid or ester (see text and Table II). [e] The literature value obtained spectrophotometrically is $(1.207 \pm 0.014) \times 10^{-4}$ s^{-1} at 98.4 °C,[4] corresponding to ca. 1.4×10^{-4} s^{-1} at 100.0 °C if $\Delta H^{\ddagger} \approx 20$ kcal/mol.[3a,b] [f] These results are slightly anomalous (see text). [g] Infinity value of peak area obtained from eq 2. [h] One data point omitted from calculation of rate constant. [i] 2,6-Di-*tert*-butylpyridine.

Table II. Least-Squares Relationships (Eq 2) between Acid and Ester Peak Areas for Average Values and First Values

	for average values[a]			first values[b]
run	P_∞/S_0	$P_\infty/10^7$	$S_0/10^7$	P_∞/S_0
1	2.676 ± 0.047	6.204 ± 0.049	2.318	2.718 ± 0.098
2	2.906 ± 0.035	6.376 ± 0.033	2.194	2.888 ± 0.059
3[c]	2.805 ± 0.067	6.115 ± 0.077	2.180	2.841 ± 0.062
4	2.742 ± 0.067	6.148 ± 0.065	2.242	2.708 ± 0.054[d]
5	2.748 ± 0.031	6.098 ± 0.027	2.219	2.693 ± 0.072
6	2.711 ± 0.050	6.179 ± 0.046	2.279	2.874 ± 0.046
7	2.774 ± 0.046	6.183 ± 0.049	2.229	2.860 ± 0.147
8	2.801 ± 0.040	6.201 ± 0.043	2.214	2.765 ± 0.081

[a] Correlation coefficient 0.999 for runs 1, 2, and 5–8, 0.998 for runs 3 and 4. [b] Correlation coefficients in order of run numbers: (1) 0.997; (2) 0.998; (3) 0.999; (4), 0.999; (5), 0.997; (6) 0.999; (7), 0.992; (8), 0.996. [c] Excluding data for the infinity point. [d] Excluding data for one "rogue point".

injections for each kinetic run (10 samples, analyzed at least in triplicate), but we can simulate what would have been achieved if only one analysis per quenched sample had been carried out. These "first values" (Table II) are in good agreement with the average values, but the statistical uncertainties are larger. Taking the worst case (run 6), we obtained a normalised result for the rate constant of $(2.24 \pm 0.03) \times 10^{-4}$ s^{-1}, probably only 3–4% in error.[7] The lower reliability of the result for run 6 is apparent from the value of P_∞/S_0 (2.874), which is significantly higher than the average of the first values of P_∞/S_0 for all eight runs (2.79) and the best estimate of P_∞/S_0 (2.76 ± 0.05, based on the range of average values excluding run 2—see Table II).

Comparison of data for runs 5–8 shows that both triethylamine and 2,6-di-*tert*-butylpyridine (2,6-DTBP) cause rate enhancements larger than expected for a weakly nucleophilic buffer (cf. urea, run 8) or a weakly nucleophilic salt (cf. run 7). Both amines appear to react with HFIP to give the hexafluoroisopropoxide. The greater reactivity of 2,6-DTBP is noteworthy; it has the same gas-phase proton affinity (231 kcal/mol) as triethylamine[8,9] but has a much lower pK_b in water.[9,10]

Conclusion

The above results illustrate the high reliability of kinetic studies by HPLC and the good accuracy attainable under relatively unfavorable circumstances.[11] There is considerable scope for extending the basic techniques outlined above to obtain other accurate rate constants, e.g.: (i) from the many and varied analyses possible by "standard" reversed-phase HPLC;[2] (ii) from analyses of non-UV-absorbing anions by chromatographic displacement of phthalate ion;[12] (iii) from immediate analyses of single aliquots of reactions in progress; (iv) from analyses of microaliquot samples (e.g., 10 × 1.8 μL), of particular importance where the substrate and/or the solvent is expensive or scarce.

Experimental Section

Chemicals. 3-(Cyclohex-1-enyl)propyl *p*-nitrobenzenesulfonate (I) was prepared and purified as described previously.[13] Hexafluoroisopropyl alcohol was distilled from and stored over 3-Å molecular sieves and the joint of storage vessel was sealed with parafilm. Triethylamine was heated under reflux with phenyl isocyanate (5% w/v) and was then distilled twice through a Vigreux column. Recrystallized urea and tetraethylammonium tetrafluoroborate (from ethanol) were available from other studies. 2,6-Di-*tert*-butylpyridine (Aldrich) was stored over 3-Å molecular sieves; the commercial sample showed no impurity peaks in GC or in the ^{1}H NMR spectrum.

Kinetics. Because of the high-vapor pressure and probable toxicity of HFIP, sealed ampules were made from Pyrex test tubes

(7) A less reliable result $(2.15 \pm 0.07) \times 10^{-4}$ was obtained from the areas of the acid peaks and a better value $(2.28 \pm 0.03) \times 10^{-4}$ from the areas of the ester peaks.

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(11) The sealed impule method reduces enthusiasm for a larger (>10) number of determinations at different reaction times. The high volatility of HFIP causes slight changes in concentration both before sealing and after unsealing the ampules. The changes in added salts and buffers, even from acidic to basic sample injections, reduces the consistency of the chromatography. The small volume injected (1.8 μL) probably increases percentage errors compared with larger injections (e.g., 5 μL).

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(10 × 75 mm), which were soaked twice in distilled water and then oven dried. Two indentations were made in the ampules, the lower one to allow a wire to grip the tube when it was in the oil bath and the higher one to permit easy sealing after charging. Kinetic runs were performed by standard procedures with 10 or 11 × ca. 0.45 mL solutions prepared in a 5-mL volumetric flask containing the ester (16.3 mg). After the kinetic run, ampules were not opened until immediately before the HPLC analysis (also the opened ampules were resealed temporarily with Teflon tape).

High-Performance Liquid Chromatography. The HPLC equipment was a Waters Solvent Metering Pump (M45), a Cecil double-beam UV detector (flow cell volume 8 μL), and an Hewlett Packard electronic integrator (HP3090A), assembled with a Rheodyne 20 μL loop injection valve and operated as described elsewhere.[14] The chromatography column (15 cm × $^1/_4$ in.) was packed with 5 μm SPERISORB ODS (Phase Separations) and had $N > 8000$ plates. Results were obtained by eluting with methanol/water (90% v/v) at 25 °C; flow rate = 1 mL/min; λ = 280 nm; absorbance range = 1.0. Injections of 1.80 ± 0.02 μL were made with a Hamilton 10 μL syringe (701 SNR), manipulated according to the manufacturers instructions for chromatographic applications. Each chromatographic separation took <3 min, the acid being eluted just before the various peaks associated with the elution of HFIP. Additives (buffers or salts) had little effect on the chromatography; tetraethylammonium tetrafluoroborate appeared to cause broadening of the acid peak and 2,6-di-*tert*-butylpyridine eluted as a separate peak of longer retention time (4.3 min). The runs (Table I) show the order in which the experiments were carried out, and the column was not used for any other purpose in the meantime.

Best values of peak areas were obtained by averaging three to five reliable values, after inspection of the HPLC trace to ensure that the "tick marks" of the electronic integrator were correctly positioned and after excluding data for a small number (ca. 1 in 20) of unreliable injections. Typically nine or ten quenched reaction mixtures in approximately equal increments of extent of reaction were analyzed in each kinetic run. The rate constants were obtained with the aid of the LSKIN computer program.[15]

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Ozonation of Phenyl-Substituted Thiophenes

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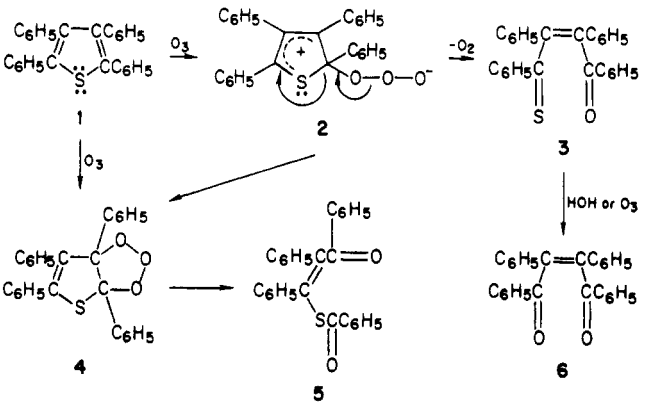
Much study has been given to the ozonation of substituted furans,[1] the most fruitful results coming from 2,5-diarylfurans[1-3] and 2,3,4,5-tetraphenylfuran.[1,4,5] When equimolar quantities of ozone and furan were employed, *cis*-butenediones were obtained as minor products in yields of 3-35%, depending on the aryl substituents, solvent, and temperature.[1] Along with this type of product, which results from electrophilic ozone attack at position 2 of the furan ring followed by loss of oxygen and 1,2-bond cleavage, products derived from 2,3-bond attack and cleavage were obtained.[1]

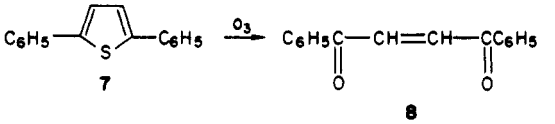
Scheme I



The ozonation of substituted pyrroles has also been given considerable study.[1] Again, the most instructive results were those from an aryl-substituted pyrrole, namely, 2,3,4,5-tetraphenylpyrrole; unfortunately, the work is reported only in the dissertation[6] and review[1] literature. The results were more complex than those from the ozonation of aryl-substituted furans, but it appears that all products can be explained by an initial electrophilic ozone attack rather than an initial bond attack.[1]

The only reported ozonations of a thiophene, before the present work, was with thiophene itself.[1] Because of the instructive studies with aryl-substituted furans and pyrroles, ozonations were performed with 2,5-diphenyl- and 2,3,4,5-tetraphenylthiophene (1). When the latter was treated with an equimolar amount of ozone at 0 °C, only 40% of the thiophene reacted; at -78 °C, only 20% of the thiophene reacted (Scheme I). From the 0 °C reaction the products, after a workup involving ethanol and in percentage yields based on unrecovered thiophene, were 1,2,3,4-tetraphenyl-2-butene-1,4-dione (6, 61%), thio ester 5 (20%), ethyl benzoate (7%), and benzoic acid (7%). The benozate and benzoic acid yields are based on an expected 2 mol of each from 1 mol of the thiophene, since approximately 2 mol of ozone per mol of thiophene actually reacted. No sulfone of 1 was detected; ozonation of the known sulfone[7] was very sluggish and yielded none of the products obtained from 1. From the -78 °C ozonation of 1, 6 was detected by TLC.

Ozonation of 2,5-diphenylthiophene (7) at 0 °C with 1 molar equiv of ozone resulted in a 46% recovery of 7 and yields of 18% and 48% of 1,4-diphenyl-2-butene-1,4-dione (8) and benzoic acid, respectively, as the only isolable products (yields again based on unrecovered 7 and an expected 2 mol of benzoic acid per mol of 7 reacting).



These results indicate that the thiophenes reacted with ozone through the same pathways as did the corresponding

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