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Note

Determination of the thermal stability of some 1,2-dioxetanes by high-performance liquid chromatography

CHARLES W. JEFFORD*, HAMISH G. GRANT, SHIGEO KOHMOTO and JOHN BOUKOUVALAS

Department of Organic Chemistry, University of Geneva, CH-1211 Geneva 4 (Switzerland)

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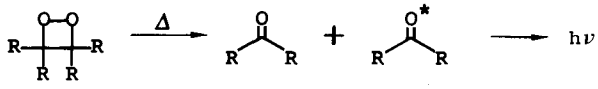
Unquestionably the single most used reaction which characterizes 1,2-dioxetanes is the thermally induced scission of the oxygen–oxygen and carbon–carbon bonds[1–6]. As one of the resulting carbonyl fragments is electronically excited, the process is usually accompanied by light emission:



Consequently, chemiluminescence has provided the basis of several methods for determining the activation parameters and stability of 1,2-dioxetanes. Despite the convenience and high sensitivity of such methods, their usefulness is often limited by practical difficulties associated with the choice of the fluorescer for energy transfer, ignorance of the real nature of the excited states involved[1,7] or more simply the availability of the requisite photometric equipment.

We were interested in the behaviour of certain 1,2-dioxetanes with respect to their propensity to undergo carbon–oxygen *vs.* oxygen–oxygen bond cleavage as the former process constitutes an avenue of reactivity[8–10]. Accordingly, we wanted to monitor their thermal stability and at the same time analyze the products of decomposition. We now report that these aims are easily achieved by the use of high-performance liquid chromatography (HPLC) which enables the activation parameters to be determined with a satisfactory accuracy.

EXPERIMENTAL

Materials and reagents

Three tricyclo[3.3.1.1^{3,7}]decane-2-spiro-3′-(4-phenoxy-1,2-dioxetanes) (4–6) were prepared by photooxygenation of the appropriate 2-phenoxymethyleneadamantanes (1–3)[8] (Fig. 1). Analytically pure samples were obtained by recrystallization from hexane at −78°C. *o*-Xylene (Fluka, puriss) was used as solvent and was treated prior to use by vigorous stirring with a 5% (v/v) solution of the disodium salt of EDTA for 12 h, followed by distillation.

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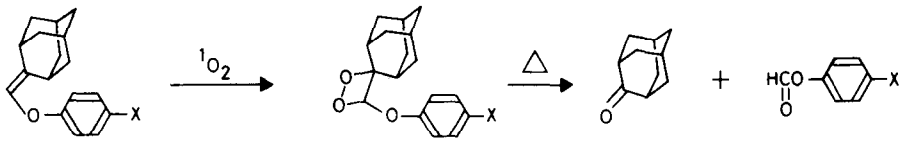


1 X = H
2 X = CH_3
3 X = OCH_3

4 X = H
5 X = CH_3
6 X = OCH_3

7 X = H
8 X = CH_3
9 X = OCH_3

Fig. 1. Preparation of dioxetanes (4–6) from enol ethers (1–3) and their thermolysis to 2-adamantanones and phenyl formates (7–9).

Apparatus

All HPLC analyses were carried out on a system consisting of a Waters M-45G pump, a Rheodyne 7125 injector equipped with a 100-μl sample loop, a Waters R401 refractive index detector, and a Hewlett-Packard 5880s integrator. A Merck LiChrosorb Si 60 column (5 μm, 25 × 0.4 cm) was used.

Thermolysis measurements

1,2-Dioxetane solutions in *o*-xylene (74 m*M*) containing 1,4-dimethoxybenzene or benzophenone (72 m*M*) as an internal standard were placed in 4-ml glass vials equipped with PTFE faced septum caps (Wilman Glass Co.) and thermoregulated ($\pm$0.2°C). The decomposition was studied in the temperature range of 50–90°C by HPLC analysis of 15-μl samples. As the solvent was the source of considerable disturbance, integration was only started after the baseline had stabilized. The HPLC conditions are indicated in Table I. At least four temperatures were used for each dioxetane, duplicate analyses were performed at each temperature.

Rate constants were determined using the standard linear regression method ($r > 0.998$ for each experiment). The activation parameters were calculated from the rate constant data by means of the Eyring equation.

TABLE I

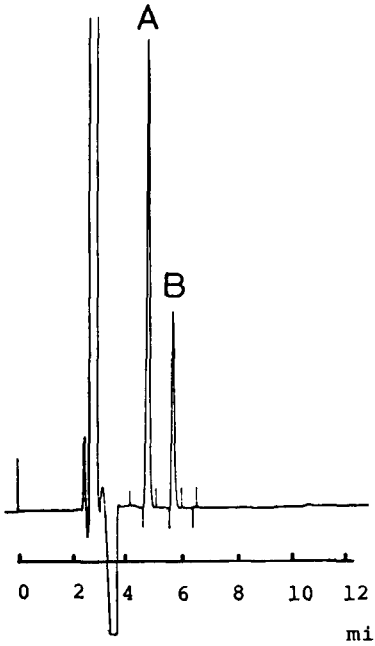
HPLC CONDITIONS AND RETENTION TIMES OF 1,2-DIOXETANES AND THEIR THERMOLYSIS PRODUCTS

Flow-rate: 0.9 ml/min.

	System for 4	*System for 5*	*System for 6*
HPLC solvent (% THF in iso-octane)	2.5	2.0	2.7
Internal standard (IS)	1,4-Dimethoxybenzene	Benzophenone	Benzophenone
Retention time (min)			
IS	5.69	7.70	6.46
1,2-Dioxetane	4.86 (4)	5.20 (5)	8.57 (6)
2-Adamantanone	10.53	13.66	9.95
Phenyl formate	6.50 (7)	7.18 (8)	11.49 (9)

RESULTS AND DISCUSSION

In order to establish suitable HPLC conditions for the analysis of the dioxetane–thermolysis product mixture, both normal silica and C_{18} bonded columns were evaluated using a range of appropriate solvents. For maximum efficiency, *i.e.*, minimum peak width, and optimum peak symmetry, it was essential to select a mobile phase that is miscible with the solvent (*o*-xylene). The system eventually chosen comprised a Merck LiChrosorb Si 60 column and isooctane–tetrahydrofuran (THF) mixtures as mobile phases (Table I). Moreover, 1,4-dimethoxybenzene or benzophenone was selected as an internal standard for the thermolysis reactions so that reproducible, quantitative data could be obtained from the chromatographs. Lastly, it was crucial to demonstrate that no significant decomposition of the dioxetanes occurred during the actual HPLC analysis. Evidence for this was supplied by the chromatograms of all three dioxetanes recorded at temperatures between 18 and 20°C, none of which revealed more than 0.25% adamantanone. Furthermore, no abnormal peak asymmetry, such as excessive tailing or formation of plateau regions, was observed (fig. 2). Hence, it is safe to conclude that there was negligible decomposition and that the theremolysis reaction could be considered as completely quenched at the time of sampling before injection onto the column. A chromatogram of the thermolysis of compound 4 is shown in Fig. 3.



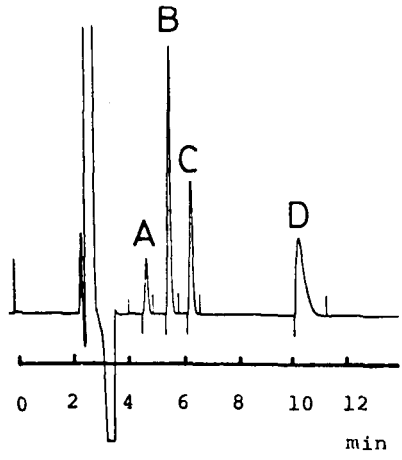
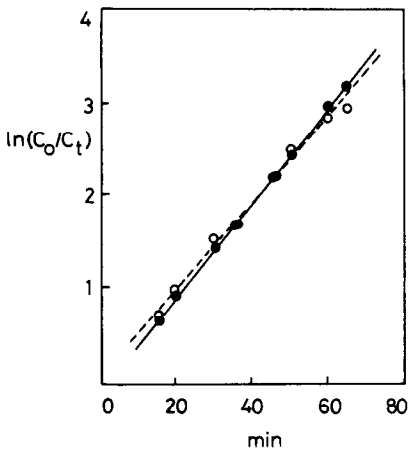


Fig. 2. HPLC chromatogram of compound 4 before thermolysis. Peaks: A = compound 4; B = internal standard.

Fig. 3. HPLC chromatogram of the products obtained from compound 4 on thermolysis at 50°C in *o*-xylene for 22.5 h. Peaks: A = compound 4; B = internal standard; C = compound 7; D = 2-adamantanone.



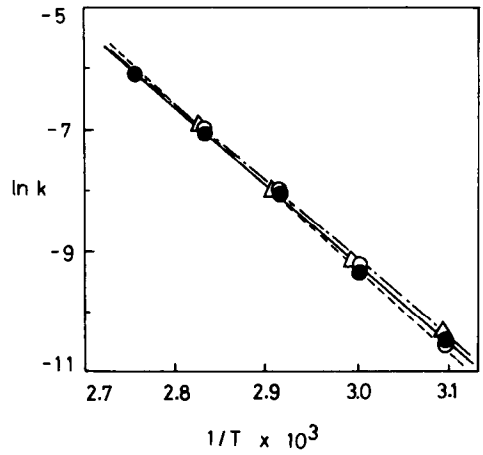


Fig. 4. The first order rate kinetics of the decrease in concentration of compound 4 (●—●, k = 8.41 · 10^{-4} s^{-1}, r = 0.9999) and the formation of 2-adamantanone (○--○, k = 8.79 · 10^{-4} s^{-1}, r = 0.9952) at 80°C.

Fig. 5. Arrhenius plots for the thermolyses of compounds 4 (●—●, r = 0.9996), 5 (○--○, r = 1.0000) and 6 (△——△, r = 0.9992).

The rate of thermolysis could be determined from either the disappearance of dioxetane or the formation of 2-adamantanone. This is well illustrated in Fig. 4 for dioxetane 4 at 80°C where the two rates are seen to be virtually identical. As expected, all rates obtained were of first order. The Arrhenius plots for the thermolysis of compounds 4–6 are shown in Fig. 5. The activation parameters $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta G^{\neq}$ obtained for compound 4 are similar to those determined by Adam *et al.*[2] using chemiluminescence assays (Table II), thereby demonstrating that an acceptable precision is offered by our method. The activation parameters of compounds 5 and 6 (Table II) show that their stability is similar to that of compound 4 in accord with their common structure.

To summarize, the results obtained show that HPLC offers a simple means of determining the thermal stability of 1,2-dioxetanes with acceptable precision. This method is a practical alternative to others based on NMR, IR spectroscopy or iodometric titration[7] and should be particularly useful for routine determinations in laboratories where photometric equipment is not available.

TABLE II

THERMODYNAMIC PARAMETERS FOR THE THERMAL DECOMPOSITION OF 1,2-DIOXETANES

1,2-Dioxetane	$\Delta H^{\neq}$ *(kcal/mol)*	$\Delta G^{\neq}$ *(kcal/mol) at 293.2 K*	$\Delta S^{\neq}$ *(cal/K · mol)*
4	25.3	25.1	0.6
	23.4 ± 0.6*	25.2*	−6 ± 0.4*
5	25.9	25.2	2.4
6	24.5	25.0	−1.5

* Value from ref. 2.

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