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Trapping of benzene oxide-oxepin and methyl-substituted derivatives with 4-phenyl- and 4-pentafluorophenyl-1,2,4-triazoline-3,5-dione

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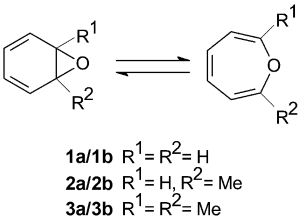
4-Phenyl-1,2,4-triazoline-3,5-dione and its pentafluoro analogue are efficient reagents for trapping arene oxides, *e.g.* benzene oxide-oxepin, affording crystalline adducts that can be quantitatively analysed by HPLC and MS techniques.

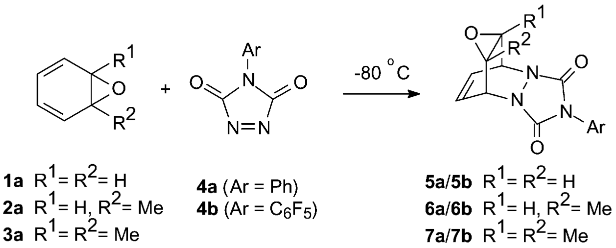
Benzene, toluene and *o*-xylene are constituents of ambient air in urban areas,[1,2] which are primarily derived from vehicle emissions. These particular 'volatile organic compounds' (VOCs) are among the most important environmental pollutants that are subject to atmospheric photo-oxidation leading to further products.[3] It has been shown *via* 'smog chamber' studies and theoretical modelling that these VOCs are highly reactive with respect to ozone formation[4] and play a significant role in the formation of secondary organic aerosols.[5] A critical issue is whether benzene oxide-oxepin **1a/b** and the methyl-substituted derivatives **2a/b** and **3a/b** (Scheme 1) are among the products of the photo-oxidation of benzene, toluene and *o*-xylene, respectively.[6] To determine whether such transient intermediates are indeed formed in the photo-oxidation of benzene and methyl-substituted benzenes, a sensitive analytical procedure is required for their detection. As the lability of **1**–**3** makes their direct analysis problematical, we considered the possibility of trapping these compounds *via* Diels–Alder reactions with their diene moieties. However, this requires a rapidly acting dienophile and conversion into stable products that can be analysed by LC/MS techniques.

We have found that 4-phenyl-1,2,4-triazoline-3,5-dione (**4a**, 'Cookson's dienophile')[7] and its 4-pentafluorophenyl analogue **4b**[8] are highly efficient reagents for trapping **1**–**3**, giving crystalline adducts that can be readily analysed by HPLC and MS techniques. **1a/b** and **2a/b** reacted exclusively *via* the benzene oxide tautomer, **1a** and **2a**. However, as found previously for reactions of **3a/b** with dienophiles (*e.g.* maleic anhydride *N*-phenyl-maleimide, 4-methyl-1,2,4-triazoline-3,5-dione),[9–11] both components, **3a** and **3b**, of the tautomeric equilibrium appear to be trapped. These observations are consistent with the known populations of the tautomeric species (*e.g.* **1a** preferred over **1b** at low temperature, **3b** strongly preferred over **3a**).[9]

Reaction of benzene oxide-oxepin **1a/b** (0.1 M) with 4-phenyl-1,2,4-triazoline-3,5-dione **4a** (0.1 M) in acetone at −80 °C gave a single adduct **5a** (Scheme 2). The structure of **5a** in solution was validated by NMR and the compound was also crystallographically characterised (Fig. 1),‡ which shows that the dienophile approaches the diene *anti* to the epoxide moiety. These data are consistent with those reported for adducts of benzene oxide with other dienophiles.[9–11] The combination of **2a/b** with **4a** gave adduct **6a** as previously reported.[12] The reaction of **3a/b** with **4a** gave two adducts, one which was the expected 1:1 adduct **7a** derived from **3a**, the structure of which was confirmed by X-ray analysis (Fig. 2)‡ and shows again the preference for *anti* addition (dienophile *vs.* epoxide). The second adduct arises from two molecules of dienophile **4a** and one molecule of **3a/3b** (Scheme 3). The structure of this adduct was elucidated as **8a** by NMR and X-ray analysis (Fig. 3).‡ Adduct **8a** was not obtained when the 1:1 adduct **7a** was incubated with dienophile **4a** and therefore appears to arise



Scheme 1 Arene oxide-oxepin tautomerism.



Scheme 2 Reactions of arene oxides with dienophile **4a** or **4b**.

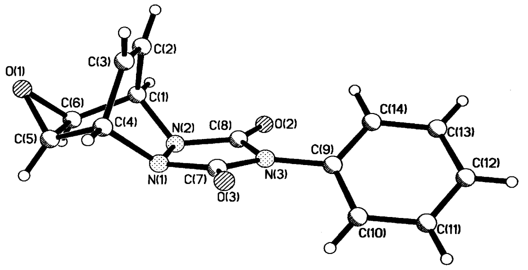


Fig. 1 Crystal structure of **5a**.

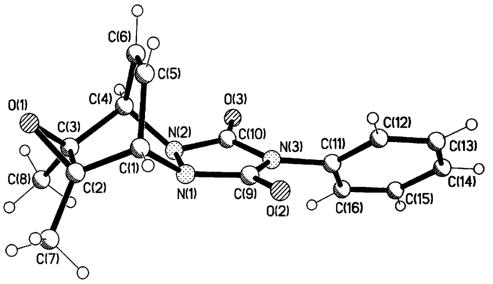
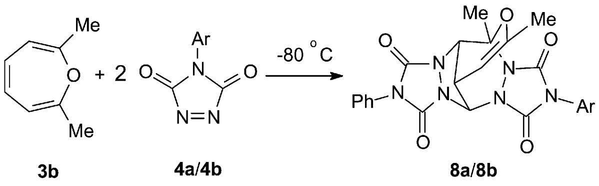


Fig. 2 Crystal structure of **7a**.



Scheme 3 Reaction of 2,7-dimethyloxepin with dienophile **4a** or **4b**.

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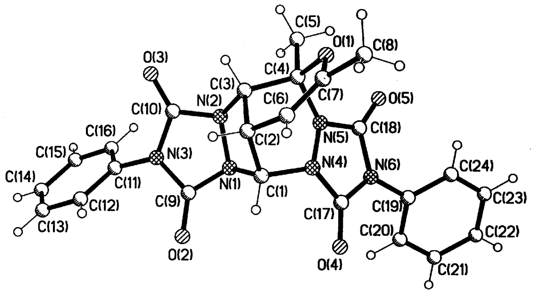

Fig. 3 Crystal structure of **8a**.

from **3b**, although a molecular rearrangement is required. The yields of adducts **7a** and **8a** were similar (1:1, total yield 55–60%) for the reaction solvents acetone and toluene, whilst for methanol the total yield was 85% (ratio of **8a**:**7a** = 5:3).

In a similar manner the pentafluorophenyl dienophile **4b** gave adducts **5b**–**8b**. The adducts **5**–**8** were all stable, crystalline substances that could be readily analysed by RP-HPLC [5 μm, 250 × 4.6 mm reversed phase C18 analytical column (Sphereclone ODS1), Phenomenex, Macclesfield, UK , isocratic elution with 30% CH_3CN/aq. ammonium formate buffer (pH 6.5) at 1 ml min^{-1}; *e.g.* R_t 11.9 min for adduct **6a**] and gave relatively intense molecular ions (*e.g.* in positive ion electron impact mass spectrometry, see footnotes).†

The reactions of dienophiles **4a** and **4b** with **1a/b**, **2a/b** and **3a/b** were extremely rapid based on the disappearance of the red colour of the dienophiles, even at −78 °C. Second order rate constants were determined using a stopped flow spectrophotometer (Applied Photophysics SX.18MV) for reactions in tetrahydrofuran at 25.2 °C using an excess of the arene oxide over the dienophile: 27 dm^3 mol^{-1} s^{-1} (reaction of **1a/1b** with **4a**) and 700 dm^3 mol^{-1} s^{-1} (reaction of **1a/1b** with **4b**). The reaction of **2a/b** with **4a** proceeded at a similar rate to that of **1a/1b**, whilst **3a/b** reacted *ca.* 2-fold slower. The similarity of the rates of reaction for **4a** shows that methyl substitution on the diene has little effect on the approach of the dienophile. The data supports the empirical observations that dienophiles **4a** and **4b** are highly efficient trapping agents for arene oxides. Remarkably, the half-life for reaction of **1a/1b** (0.01 mol dm^{-3}) with **4b** (0.01 mol dm^{-3}) is 0.1 s.

The results described show that dienophiles **4a** and **4b** rapidly and efficiently trap dienes **1**–**3**, to give adducts (**5**–**8**) suitable for quantitative analysis. Applications of dienophile **4b** to the trapping of arene oxides in 'smog chambers' are in progress. Arene oxides have also been shown to arise from the metabolism of arenes by cytochrome P450.[13] The methodology described may be applicable to the quantitative detection of arene oxide metabolites in both *in vitro* and *in vivo* experiments.

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Notes and references

‡ *Crystal data*: for **5a**: $C_{14}H_{11}N_3O_3$, M = 269.3, orthorhombic, space group *Pbca*, a = 10.8897(7), b = 14.2214(9), c = 15.2607(10) Å, U = 2363.4(3) $Å^3$, Z = 8, D_c = 1.513 g cm^{-3}, μ = 0.11 mm^{-1} (Mo-Kα, λ = 0.71073 Å), T = 160 K, $R(F^2 > 2\sigma F)$ = 0.0383, $R_w(F^2$, all data) = 0.1059 for 2845 unique data (17468 measured, R_{int} = 0.0264, $2\theta < 28.6°$, CCD diffractometer) and 181 refined parameters.

For **7a**: $C_{16}H_{15}N_3O_3$, M = 297.3, orthorhombic, space group *Pna*2_1, a = 13.5339(10), b = 11.3119(8), c = 9.3316(7) Å, U = 1428.6(2) $Å^3$, Z = 4, D_c = 1.382 g cm^{-3}, μ = 0.10 mm^{-1} (Mo-Kα, λ = 0.71073 Å), T = 160 K, $R(F^2 > 2\sigma F)$ = 0.0388, $R_w(F^2$, all data) = 0.0935 for 1767 unique data (7985 measured, R_{int} = 0.0318, $2\theta < 28.3°$) and 202 refined parameters.

For **8a**·$2CH_2Cl_2$: $C_{26}H_{24}Cl_4N_6O_5$, M = 642.3, triclinic, space group *P*1, a = 9.1142(8), b = 9.5955(9), c = 17.0216(15) Å, α = 85.792(2), β = 78.365(2), γ = 74.561(2)°, U = 1405.1(2) $Å^3$, Z = 2, D_c = 1.518 g cm^{-3}, μ = 0.47 mm^{-1} (Mo-Kα), T = 160 K, $R(F^2 > 2\sigma)$ = 0.0493, $R_w(F^2$, all data) = 0.1434 for 6313 unique data (12071 measured, R_{int} = 0.0339, $2\theta < 28.4°$) and 392 refined parameters. The structure contains ordered and disordered solvent molecules.

Programs: standard Bruker ASX control and integration software and SHELXTL. CCDC reference numbers 188253–188255. See http://www.rsc.org/suppdata/cc/b2/b205079j/ for crystallographic data in CIF or other electronic format.

† *Mass spectral data* for **5a**: MS (+EI) *m/z* 269 (M^+, 58 %), 240 (29), 94 (100), 77 (39).

For **6a**: MS (+EI) *m/z* 283 (M^+, 29%), 240 (100), 108 (13), 91 (14), 78 (27).

For **7a**: MS (+EI) *m/z* 297 (M^+, 100%), 254 (90), 227 (22), 220 (13), 77 (28).

For **8a**: MS (+EI) *m/z* 472 (M^+, 100 %), 429 (28), 220 (8), 119 (22), 109 (35), 78 (10).

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