Chemiluminescence of the Labile 1,2-Dioxetanes and Epoxides Produced in the Oxidation of *N*-Acetylated Dihydro- and Tetrahydropyrazines by Singlet Oxygen, Dimethyldioxirane, and *m*-Chloroperoxybenzoic Acid

Waldemar Adam,* Michael Ahrweiler, and Pavel Vlček

Contribution from the Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

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Abstract: The oxidation of the *N*-acylated pyrazine derivatives **1a,b** gave the pyrazine-type dioxetanes **2a,b**, which for the first time were isolated and characterized. The halflives of the thermal decomposition of these labile dioxetanes at 20 °C were determined by chemiluminescence measurements to be 80 ± 2 (**2a**) and 18 ± 1 min (**2b**). Upon thermolysis, the dioxetanes **2a,b** decomposed quantitatively to the corresponding C_2-C_3 cleavage products **3a,b**. The deoxygenation of the dioxetanes **2a,b** by dimethyl sulfide yielded predominantly the novel pyrazine-type epoxides **4a,b**, accompanied by some dioxetane decomposition product **3a,b**. Upon thermolysis, the epoxide **4b** decomposed to the benzodiazine **5b** and the enone **6b**. Dimethyldioxirane oxidation of the pyrazine derivatives **1a,b** afforded the epoxides **4a,b**, while treatment with an excess of *m*-chloroperoxybenzoic acid (*m*-CPBA) led to the C_2-C_3 cleavage products **3a,b** with intense light emission by way of the intermediary peroxy esters **8**.

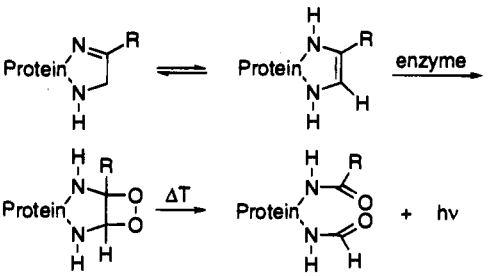
Introduction

The oxidative metabolism of arenes and heteroarenes is a major pathway of their enzymatic detoxification in the cell.[1] Nonetheless, arenes are metabolized by cytochrome P-450 monooxygenase to the corresponding arene oxides,[2] which constitute highly reactive epoxides that initiate carcinogenesis through DNA alkylation.[3] Additionally, there exists suggestive evidence that 1,2-dioxetanes are involved as potential intermediates in the oxidative metabolism.[4] For example, in the microsomal cytochrome P-450 oxidation of 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene,[5] as well as in the heme-catalyzed aerobic metabolization of Schiff bases of proteins and saccharides, chemiluminescence was observed (Scheme 1).[6] In the latter case, presumably α-nitrogen-substituted 1,2-dioxetanes serve as intermediates.[4,6]

Generally, such nitrogen-containing dioxetanes are thermally too labile to be isolated since they decompose readily[7] by the chemical induced electron exchange (CIEEL),[8] initiated by electron transfer from the lone pair of the nitrogen atom. These labile transients have been observed spectrally only in a few cases at low temperatures.[7b,c,e,8] In the case of the labile indole dioxetanes,[9] the latter could be sufficiently stabilized by acylation of the nitrogen atom to allow not only their spectral characterization[10] but also their isolation.[10d-f] Since indoles and other heteroarenes like pyrroles, imidazoles, and pyrazines have been proposed as cofactors in chemical carcinogenesis,[1] it is quite likely that their epoxides and dioxetanes, produced by enzymatic oxygenation,[1b] are engaged in the DNA damage.

Recently, a dioxetane was claimed to have been isolated in the air oxidation of a substituted 1,4-dihydropyrazine.[11] Other than this report, no authentic pyrazine-type dioxetanes appear

Scheme 1



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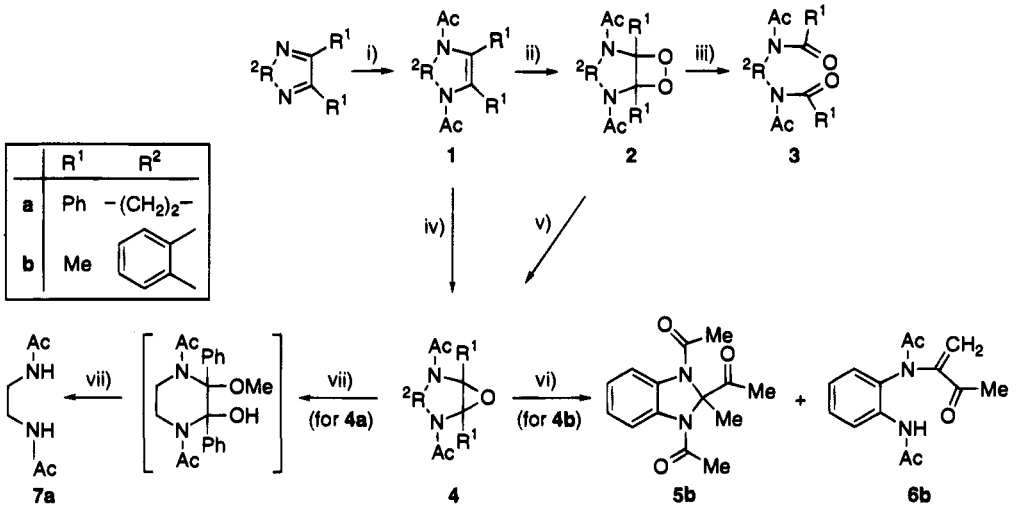
Scheme 2



i) Ac_2O, Zn, 110 °C, 12-72 h; ii) O_2, sens., hv, CH_2Cl_2, -20 °C; iii) CD_2Cl_2, 20 °C, 24 h; iv) , acetone, 0 °C, 6-48 h; v) Me_2S, $CDCl_3$, 0 °C, 2 h; vi) silica gel, ethyl acetate, 20 °C, 7 d; vii) CF_3CO_2H, MeOH, -20 °C, 1 d; viii) silica gel, Et_2O, 0 °C, 15 min.

to be known and we decided to examine the photooxygenation of *N*-acylated pyrazine derivatives **1** (Scheme 2). Moreover, since nitrogen-substituted epoxides are also labile entities,[12] which have been recently observed through stabilization by *N*-acylation,[10] such highly reactive oxidative metabolites may serve as effective DNA-damaging agents on account of their alkylating propensity. Thus, it was also our interest to prepare authentic pyrazine-type epoxides of the *N*-acylated derivatives **4** by dimethyldioxirane or *m*-CPBA and establish the chemical groundwork for such biological activity. We succeeded in preparing and characterizing the first dioxetane **2** and epoxide **4** representatives of the *N*-acylated pyrazines **1** (Scheme 2) and present herein the results of our unprecedented oxidation studies.

Results and Discussion

1,4-Diacetyl-2,3-diphenyl-1,4,5,6-tetrahydropyrazine (**1a**) was prepared as described by Fowler[13c] from the reaction of 2,3-diphenylpyrazine with acetic anhydride and zinc (Scheme 2). Analogously, the unknown pyrazine **1b** was obtained from 2,3-dimethylquinoxaline[13] in 68% yield. The proposed structure for the latter is based on its characteristic IR and NMR data, as well as a satisfactory elemental analysis (cf. Experimental Section, supporting information).

The photooxygenation of the *N*-acyl pyrazines **1a,b** in dichloromethane or deuteriochloroform at −20 to −10 °C gave mainly the desired dioxetanes **2a,b** and the corresponding cleavage products **3a,b** (Scheme 2). The dioxetane **2a** was isolated in 37% and dioxetane **2b** in 23% yields by low-temperature silica gel chromatography, both accompanied by 5−15% of the corresponding cleavage products **3** from thermal decomposition of these labile dioxetanes.[8] The characteristic spectral data of the dioxetanes **2a,b** are similar to those reported for the indole dioxetanes.[10] Additionally, direct chemiluminescence as well as a positive peroxide test (HOAc/KI) and chemical transformations (Scheme 2) confirm the proposed structure. The dioxetanes **2a,b** decomposed at 20 °C with intense chemiluminescence ($t_{1/2}$ 80 min for **2a** and 18 min for **2b**) quantitatively to the expected C_2-C_3 cleavage products **3a,b** (Scheme 2), which were fully characterized.

These novel results clearly demonstrate the thermal lability of the pyrazine dioxetanes **2a,b** even in their *N*-acylated form. Thus, the suggested structural assignment of the reported pyrazine dioxetane[11] is questionable since the characteristic spectral and chemical evidence is missing. The reported data fit much better the corresponding dioxetane cleavage product. Be this as it may, analogous to the indole dioxetanes,[10] acylation of the nitrogen atom stabilizes the corresponding pyrazine dioxetanes **2a,b** sufficiently to permit their isolation and structural characterization.

Deoxygenation of the dioxetane **2a** by dimethyl sulfide (1 equiv)[14] at 0 °C gave the unknown pyrazine epoxide **4a** and the cleavage product **3a** in a ratio of 71:29 (Scheme 2). Analogously, upon treatment of the dioxetane **2b** with equimolar amounts of dimethyl sulfide, the corresponding epoxide **4b** was detected by NMR spectroscopy. Silica gel chromatography of the reaction mixture yielded 35% of the cleavage product **3b**, 24% of the benzodiazine **5**, and 16% of the enone **6b**. The structure assignment of the benzodiazine **5b** and the enone **6b** rests on their characteristic analytical data (cf. Experimental Section).

The epoxide **4a** was isolated in 94% yield by epoxidation of the pyrazine **1a** with an excess (5 equiv) of dimethyldioxirane[15] and was fully characterized. As a side product, also the corresponding dioxetane decomposition product **3a** was observed in 3% yield by ^1H NMR spectroscopy on the crude product mixture. The formation of the cleavage product **3a** may be rationalized by further oxidation of epoxide **4a** in analogy to examples reported previously.[16] In contrast, the reaction of

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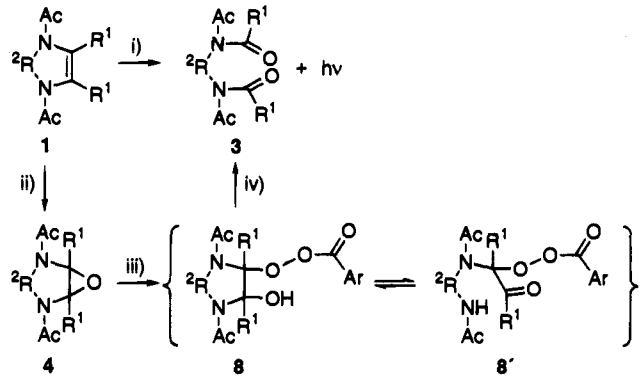
Scheme 3



i) *m*-CPBA (2 equiv.), CH_2Cl_2, 0 °C, 8-24 h; ii) and iii) *m*-CPBA (1 equiv.), $CDCl_3$, 0 °C, 8 h; iv) CH_2Cl_2, 0 °C, 1d; for R^1 and R^2 cf. Scheme 2.

the pyrazine **1b** with an excess (5 equiv) of dimethyldioxirane was much slower (only 50% conversion after 48 h at 0 °C) and the epoxide **4b** was detected as sole product as determined by NMR spectroscopy. Several attempts to purify the labile epoxide **4b** by silica gel chromatography or recrystallization failed. Nevertheless, the structures of the epoxides **4a,b** were rigorously established by comparison of the characteristic NMR data with those of the related indole epoxides[10] and on the basis of their chemical transformations (Scheme 2).

In view of the high reactivity of nitrogen-substituted epoxides derived from enamines[12] and indoles[10c] toward nucleophiles, as expected, the epoxide **4a** was readily trapped with an excess (50-fold) of methanol by acid-catalyzed epoxide opening. However, the corresponding methanol adduct could not be detected even by low-temperature NMR spectroscopy because it readily transformed to the known *N,N*-diacyldiaminoethane **7a**.[17]

Upon treatment of the pyrazine derivatives **1a,b** with 2 equiv of *m*-CPBA at 0 °C, the dioxetane cleavage products **3a,b** were formed quantitatively (Scheme 3). In the case of substrate **1a**, the epoxide **4a** was identified by NMR spectroscopy. The reaction of epoxides **4a,b** with 1 equiv of *m*-CPBA at −20 °C resulted in the intermediary peroxy esters **8a,b** and their respective ring-opened tautomers **8a′,b′**. The peroxy ester **8a′** was observed by low-temperature NMR spectroscopy and its structure convincingly established by the characteristic NMR resonances (cf. Experimental Section). Upon thermolysis at 0 °C, the peroxy esters **8a,b** yielded quantitatively the cleavage products **3a,b** under emission of chemiluminescence. The emitting species on the reaction of epoxides **4a,b** with *m*-CPBA is probably the singlet excited state of diimides **3a,b**. The mechanism of the formation of the cleavage products **3a,b** is similar to that proposed in the *m*-CPBA oxidation of indoles[18a,b] and benzofurans,[18c] i.e. Grob fragmentation of the intermediary peroxy esters **8** (Scheme 3). Analogous chemiluminescence was previously observed in the *m*-CPBA oxidation of 9-methyleneacridines.[18d] Such chemiluminescent peroxide fragmentations, which do not involve intermediary dioxetanes, are still quite rare.

In summary, we have shown that deactivation of the nitrogen lone pair by acylation arrests its CIEEL activity and makes possible the isolation and characterization of the sufficiently persistent pyrazine-type dioxetanes **2a,b** in the photooxygenation of the pyrazine derivatives **1a,b**. On thermolysis, the dioxetanes were quantitatively transformed into the cleavage products **3a,b**, whereas deoxygenation of the dioxetane **2a** by dimethyl sulfide led to the epoxide **4a**. Independently, the epoxides **4a,b** were prepared in good yields by dimethyldioxirane oxidation of the corresponding pyrazine derivatives **1a,b**. In contrast, the oxidation of the substrates **1a,b** with 2 equiv of *m*-CPBA resulted quantitatively in the cleavage products **3a,b** with light emission. Biological tests[19] on the authentic pyrazine dioxetanes **2a,b** and epoxides **4a,b** should be of interest to evaluate their genotoxic potential.

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Supporting Information Available: Experimental procedures, including synthesis and characterization data of all new compounds (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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