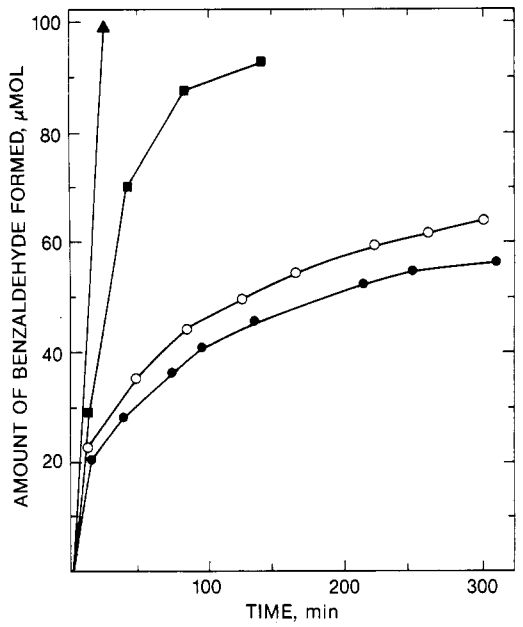
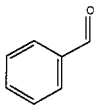
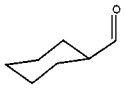


Figure 1. Comparison of coenzymes for the oxidation of benzyl alcohol to benzaldehyde catalyzed by HLADH. Oxidations using $APAD^+$ (▲) or $SNAD^+$ (■) are faster than those with NAD^+ (●). Conditions: benzyl alcohol (100 μmol, 10 mM) in TES buffer (50 mM, pH 8), coenzyme (1 mM), bis(ammonium) 2-oxoglutarate (200 mM), alcohol dehydrogenase (10 units, based on ethanol/NAD^+), glutamate dehydrogenase (50 units). Dilution of the NAD^+-containing reaction by a factor of two (○) also shows faster formation of benzaldehyde consistent with the notion that inhibition by benzaldehyde limits the rate of reaction.

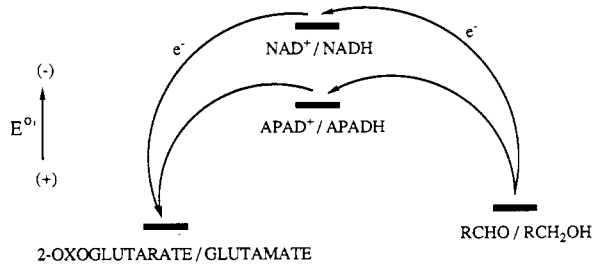
Table II. Oxidation of Other Alcohols Catalyzed by HLADH

product		coenzyme NAD⁺	SNAD⁺	APAD⁺
	K_i,[a] mM	0.10	1.4	3.1
	V_{max},[a] %	90	20	500
	synthesis,[b] % conversion	45	93	100
	K_i,[c] mM	3.7	150	600
	V_{max},[c] %	120	210	930
	synthesis,[d] % conversion	25	55	62
	K_i,[e] mM	0.40	2.5	16
	V_{max},[e] %	20	70	300
	synthesis,[e,f] % conversion	5	nd	22
	K_i,[g] mM	190	250	400
	V_{max},[g] %	90	140	120

[a] 2 mM benzyl alcohol, 50 mM TES buffer, pH 8, 25 °C (TES = *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid). K_i is the inhibition constant for the product aldehyde. V_{max} is the relative rate of oxidation as compared to ethanol at pH 9, 25 °C. [b] Data in cation of Figure 1. [c] 30 mM 2-methylprop-2-en-1-ol, 50 mM TES buffer pH 8, 25 °C. [d] 100 mM 2-methylprop-2-en-1-ol, cofactor was regenerated as in caption of Figure 1. [e] 20 mM cyclohexanemethanol, 0.1 M borate buffer, pH 9, 25 °C. [f] Cofactor was regenerated with methylene blue (1 mM), catalase (0.1 mg/mL), 400-μmol scale, HLADH (1.2 units), 0.4 mM coenzyme. [g] 0.2 M cyclohexanol, 0.1 M borate buffer, pH 9.0, 25 °C.

NAD^+ (analogues where the adenine portion was modified) showed no improvement. Further, oxidation of cyclohexanol, which is thermodynamically easier that the oxidation of a primary alcohol,[5] showed (1) little product inhibition by cyclohexanone and (2) little change in product inhibition upon substitution of NAD^+ with $SNAD^+$ or $APAD^+$ (Table II).

Scheme I[a]



[a] The free energy of the intermediate electron carrier is lowered upon substitution of $APAD^+$ for NAD^+ in an oxidation of an aldehyde by ammonium 2-oxoglutarate.

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Synthesis and Chemiluminescence of an α-Hydroperoxyacyl Cyanide

Summary: An isolable enol (**3**) reacts with triplet dioxygen to afford an α-hydroperoxyacyl cyanide (**4**); treatment of **4** with base provides evidence for the intermediacy of a 1,2-dioxetan-3-one (**5**).

Sir: Since α-peroxy lactones (1,2-dioxetanones) were first suggested as key intermediates in bioluminescence,[1] they have been synthesized[2] and the chemienergization processes underlying their luminescence have become fairly well understood.[3] In this paper we report a new light-producing reaction, presumably involving α-peroxy lactones.

Treatment of isobutyryl cyanide[4] (2-oxo-3-methylbutanenitrile, **1**) with triethylamine and a large excess of trifluoroacetic anhydride led to formation of the enol

(1) Hopkins, T. A.; Seliger, H. H.; White, E. H.; Cass, M. W. *J. Am. Chem. Soc.* **1967**, *89*, 7148-7150. McCapra, F.; Chang, Y. C.; Francois, V. P. *J. Chem. Soc., Chem. Commun.* **1968**, 22-23. McCapra, F. *Pure Appl. Chem.* **1970**, *24*, 611-629. White, E. H.; Rapaport, E.; Seliger, H. H.; Hopkins, T. A. *Bioorg. Chem.* **1971**, *1*, 92-122.

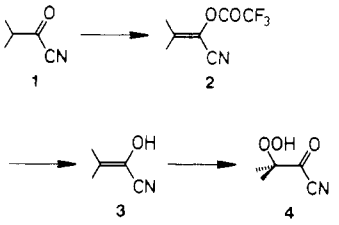
(2) Adam, W.; Liu, J.-C. *J. Am. Chem. Soc.* **1972**, *94*, 2894-2895. Adam, W.; Steinmetzer, H.-C. *Angew. Chem.* **1972**, *84*, 590-592. Adam, W.; Alzérreca, A.; Liu, J.-C.; Yany, F. *J. Am. Chem. Soc.* **1977**, *99*, 5768-5773. Turro, N. J.; Ito, Y.; Chow, M.-F.; Adam, W.; Rodriquez, O.; Yany, F. *Ibid.* **1977**, *99*, 5836-5838. Adam, W. *Chem. Heterocycl. Comp.* **1985**, *42*, 351-429.

(3) Schmidt, S. P.; Schuster, G. B. *J. Am. Chem. Soc.* **1980**, *102*, 306-314. Adam, W.; Yany, F. *Photochem. Photobiol.* **1980**, *31*, 267-272. Turro, N. J.; Chow, M.-F. *J. Am. Chem. Soc.* **1980**, *102*, 5058-5064. Schmidt, S. P.; Vincent, M. A.; Dykstra, C. E.; Schuster, G. B. *Ibid.* **1981**, *103*, 1292-1293. Schuster, G. B.; Schmidt, S. P. *Adv. Phys. Org. Chem.* **1982**, *18*, 187-238. *Chemical and Biological Generation of Excited States*; Adam, W., Cilento, G., Eds.; Academic: New York, 1982.

(4) Clement, B. A.; Soulen, R. L. *J. Org. Chem.* **1974**, *39*, 97-98.

0022-3263/88/1953-4635$01.50/0

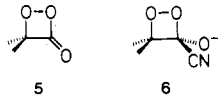
trifluoroacetate **2** in quantitative yield.[5,6] Solvolysis of **2** in neat methanol at room temperature was practically instantaneous and yielded **3**,[7] the crystalline metastable enol of **1**, a representative of a little-known class of isolable enols.[8] The enol was stable toward trifluoroacetic acid



overnight, but immediately gave isobutyric acid or methyl isobutyrate with aqueous or methanolic base, respectively. Aeration of solutions of **3** in nonpolar solvents (benzene, dichloromethane, chloroform) at room temperature caused rapid oxygen uptake[9] with formation of hydroperoxide **4**,[10] the first α-hydroperoxyacyl cyanide to be obtained. The hydroperoxide was stable at room temperature in solution for at least several hours (loss of no more than 5% of the iodometric titer), being apparently stable indefinitely at −20 °C.

Treatment of a solution of **4** (0.01–0.1 M) with triethylamine or 1,4-diazabicyclo[2.2.2]octane (0.1–1 equiv) caused an exothermic reaction accompanied by easily seen luminescence, which was greatly enhanced by addition of rubrene, 9,10-dibromoanthracene, or, in particular, 9,10-diphenylanthracene. In the absence of fluorescers, the emitted radiation possessed the characteristics of acetone fluorescence (λ_{max} 400 nm, uncorrected), becoming stronger and red-shifted on deoxygenation of the solution, presumably as a result of a contribution from acetone phosphorescence.[11] The decrease of the light emission with time (in the presence of 9,10-diphenylanthracene) and the loss of oxidizing power (determined iodometrically) showed first-order kinetics with a half-life of 4 min at ambient temperature. Acetone (ν_{max} 1711 cm^{-1}, yield about 90% by ^{1}H NMR), carbon dioxide (ν_{max} 2337 cm^{-1}), and hydrogen cyanide (ν_{max} 2092 cm^{-1}; all spectra in CH_2Cl_2) were formed.

We were not able to detect **5** by its characteristic[2] IR absorption band, presumably because its decomposition was fast compared to the cyclization of **4**. Chemiluminescent decomposition of the intermediate **6** to acetone and cyanoformate is a yet untested alternative to the involvement of **5**. The light-emitting solutions of **4** showed,



besides the growing acetone carbonyl band, another carbonyl absorption at 1785 cm^{-1} (CH_2Cl_2), which we assign to polyperesters[12,13] formed in bimolecular reactions; this band was formed especially in more concentrated solutions and was apparently unrelated to the light emission, persisting after the chemiluminescence had ceased. Such solutions still contained about 10% of the original iodometric titer and become weakly chemiluminescent upon heating in the presence of fluorescers.

Dioxetanones are generally formed by cyclization of precursors possessing a hydroperoxy group next to an active acyl group. Thus in the case of firefly luciferin a mixed carboxylic phosphoric anhydride serves as an intramolecular acylating agent,[1,14] whereas synthetically use is made of the activation of the carboxyl group in α-hydroperoxy acids by DCC.[2] The yield of α-peroxy lactones by cyclization of α-hydroperoxides of unactivated alkyl ester is low.[15] In the present synthetic scheme the nitrile function is used to stabilize an enol group, which upon reaction with triplet oxygen becomes transformed into an activated acyl group.[16] All compounds described here can be readily handled at ambient conditions by ordinary techniques; by contrast, synthesis of other dioxetanone precursors involves low-temperature carbanion technology or singlet oxygen chemistry.[2,17] We believe that the present synthetic route may be useful for synthesis or at least in situ generation of α-peroxy lactones with large, highly oxidizable substituents, capable of decomposing via the intramolecular CIEEL mechanism[18] to efficiently emitting, excited singlet states, possibly affording better models of natural bioluminescent systems than those available today.

Acknowledgment. Financial support to J.W.J. from the Danish Natural Science Research Council is gratefully acknowledged. We thank Professor O. Buchardt, De-

(5) The enol trifluoroacetate was isolated by vacuum distillation: mp −2 °C; bp 40 °C (ca. 3 mmHg); UV (CH_3CN) λ_{max} 213 nm (ϵ 10 000); ^{1}H NMR ($CDCl_3$) δ 1.89 and 2.15; ^{13}C NMR ($CDCl_3$) δ 18.0, 20.8, 111.9, 114.5 (q, $^2J_{CF}$ = 285.5 Hz), 116.4, 147.4, 154.7 (q, $^3J_{CF}$ = 45 Hz); ^{19}F NMR ($CDCl_3$) singlet 0.63 ppm downfield from internal CF_3COOCH_3; IR (film) 2225 (s), 1811 (s), 1665 (m) cm^{-1}; mass spectrum (70 eV), *m/e* 193 (25, M), 70 (100), 69 (60). Anal. ($C_7H_6F_3NO_2$) C, H, N.

(6) Replacement of trifluoroacetic anhydride with acetyl or pivaloyl chloride afforded the corresponding enol esters. Pivalate: ^{1}H NMR ($CDCl_3$) δ 1.30, 1.78, 2.07; ^{13}C NMR ($CDCl_3$) δ 17.6, 20.6, 26.3, 39.0, 113.5, 117.6, 143.6, 175.4; IR (film) 2222 (s), 1763 (s), 1665 (m) cm^{-1}. Acetate: cf. Jaroszewski, J. W.; Ettlinger, M. G. *Magn. Reson. Chem.* **1987**, *25*, 555–557. Addition of triethylamine to neat **1** gave the enol isobutyrate; cf.: Oku, A.; Nakaoji, S.; Kadono, T.; Imai, H. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2966–2969. See also: Hertenstein, U.; Hünig, S.; Reichelt, H.; Schaller, R. *Chem. Ber.* **1986**, *119*, 699–721.

(7) Mp 30–32 °C (recrystallized from pentane); UV (CH_3OH) λ_{max} 226 nm (ϵ 10 600); ^{1}H NMR ($CDCl_3$) δ 1.83, 1.92, and 5.25 (br); ^{13}C NMR (CD_3OD) δ 16.6, 20.2, 116.8, 121.8, 129.7; IR (CH_2Cl_2) 3560 (s, sharp), 3345 (s, br), 2220 (s), 1669 (m), 1665 (m; this second C=C stretching band become increasingly weaker upon dilution of the solution and is thus presumably due to hydrogen-bonded molecules) cm^{-1}.

(8) Cf.: McDonald, R. N.; Hill, D. G. *J. Chem. Soc., Chem. Commun.* **1969**, 671–672. Kametani, T.; Kigasawa, K.; Hiiragi, M.; Ishimaru, H.; Haga, S.; Shirayama, K. *J. Pharm. Soc. Jpn.* **1978**, *98*, 146–152. Kametani, T.; Kigasawa, K.; Hiiragi, M.; Ishimaru, H.; Haga, S.; Shirayama, K. *J. Heterocycl. Chem.* **1978**, *15*, 369–375. Voronkov, M. G.; Keiko, N. A.; Kuznetsova, T. A.; Pestunovich, V. A.; Tsetlina, E. O.; Keiko, V. V. *J. Gen. Chem. U.S.S.R.* **1980**, *49*, 2199–2222. Voronkov, M. G.; Keiko, N. A.; Kuznetsova, T. A.; Pestunovich, V. A.; Kalikhman, I. D. *Doklady Chem.* **1980**, *247*, 329–331. Voronkov, M. G.; Keiko, N. A.; Kuznetsova, T. A.; Tsetlina, E. O.; Pestunovich, V. A.; Pogodaeva, T. K. *J. Org. Chem. U.S.S.R.* **1980**, *16*, 241–243.

(9) Enols have long been known to react readily with triplet oxygen under neutral conditions: Attenburrow, J.; Connett, J. E.; Graham, W.; Oughton, J. F.; Ritchie, A. C.; Wilkinson, P. A. *J. Chem. Soc.* **1961**, 4547–4559. Pinkus, A. G.; Hag, M. Z.; Lindberg, J. G. *J. Org. Chem.* **1970**, *35*, 2555–2559. Enslin, P. R. *Tetrahedron* **1971**, *27*, 1909–1915.

(10) IR (CH_2Cl_2) 3510 (m), 2222.5 (m), 1733 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50; ^{13}C NMR ($CDCl_3$) δ 19.8, 87.3, 112.2, 179.8. The yield based on **2** was over 90% (iodometric titration).

(11) Cf.: Turro, N. J.; Steinmetzer, H.-C.; Yekta, A. *J. Am. Chem. Soc.* **1973**, *95*, 6468–6470. The increase of emission intensity was less than in the case of photoexcited acetone, triplet quenchers other than oxygen being apparently present in the solution.

(12) Dixon, B. G.; Schuster, G. B. *J. Am. Chem. Soc.* **1981**, *103*, 3068–3077.

(13) Turro, N. J.; Chow, M.-F.; Ito, Y. *J. Am. Chem. Soc.* **1978**, *100*, 5580–5582.

(14) White, E. H.; Steinmetz, M. G.; Miano, J. D.; Wildes, P. D.; Morland, R. *J. Am. Chem. Soc.* **1980**, *102*, 3199–3208.

(15) Sawaki, Y.; Ogata, Y. *J. Org. Chem.* **1977**, *42*, 40–44.

(16) Cf. the acylating properties of acyl cyanides: Hünig, S.; Schaller, R. *Angew. Chem.* **1982**, *94*, 1–27.

(17) Adam. W.; Cueto, O.; Ehrig, V. *J. Org. Chem.* **1976**, *41*, 370–371.

(18) Koo, J.-Y.; Schmidt, S. P.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 30–33. Schaap, A. P.; Gagnon, S. D. *J. Am. Chem. Soc.* **1982**, *104*, 3504–3506.

partment of General and Organic Chemistry, University of Copenhagen, for loan of a Hitachi MPF-3 fluorescence spectrophotometer used in this work.

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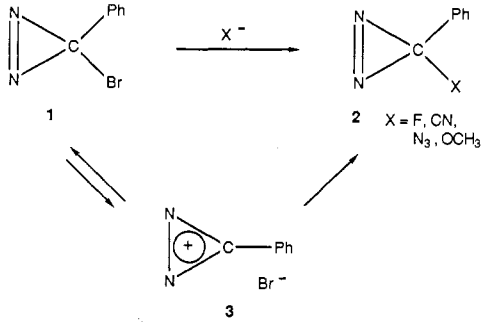
On the Intermediacy of Phenyldiazirinium Cation in Olefin Isomerizations

Summary: The phenyldiazirinium cation cannot be easily generated under solvolytic conditions. This study conflicts with the reported determination of an equilibrium constant for ionic dissociation of 3-bromo-3-phenyldiazirine while further studies indicate that the phenyldiazirinium cation is not involved in the diethyl maleate to fumarate isomerization as previously reported.

Sir: During the course of studies designed to prepare substituted methylenecyclopropanes, we wanted to prepare a series of substituted diazirines to use as cyclopropane precursors. We therefore prepared 3-bromo-3-phenyldiazirine, **1**, which is known to undergo reaction with certain nucleophiles to give the novel and interesting substitution products **2**.[1] It has been suggested that these products result from nucleophilic capture of a reversibly formed ion pair **3**.[1a] Subsequently, measurement of the equilibrium constant for dissociation of **1** in acetonitrile by a conductometric technique was reported.[2] It was also reported that **1** catalyzes the isomerization of diethyl maleate to diethyl fumarate, and it was further suggested that the ion pair **3** was the catalytic agent.[3] We now report studies that call into question the involvement of cation **3** in the maleate to fumarate isomerization.



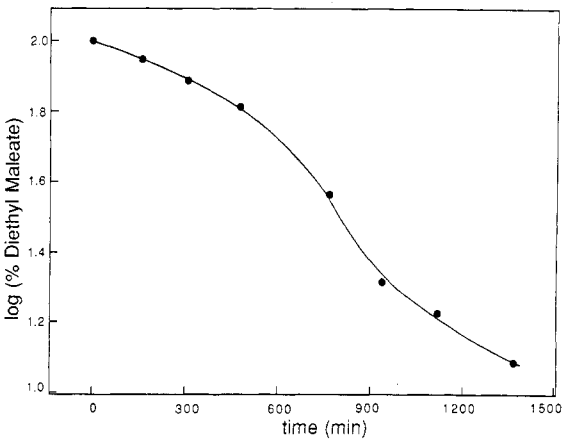
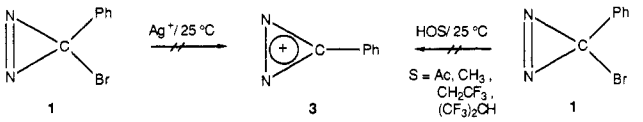


Figure 1. A plot of log (% unreacted diethyl maleate) vs time for isomerization of a 0.57 M solution of diethyl maleate in CCl_4 containing 0.13 M diazirine **1** at room temperature.

In an attempt to form the nitrate derivative, the bromodiazirine **1** was reacted with silver nitrate in acetonitrile. To our surprise the mixture only turned cloudy. There was no instantaneous copious precipitation of silver bromide as when triphenylmethyl bromide or benzyl bromide was reacted under the same conditions in acetonitrile.[4] This observation is inconsistent with the report that **1** is sufficiently ionized in acetonitrile to allow conductometric measurements.[5] Attempts to generate the cation **3** under solvolytic conditions were also unsuccessful. The bromodiazirine **1** was recovered unchanged after standing in acetic acid for 20 h at room temperature. The bromodiazirine **1** was also unreactive with silver acetate in acetic acid. In neutral methanol, **1** was unreactive over a period of 20 h. Bromodiazirine **1** was also recovered unchanged on standing in the more highly ionizing solvent trifluoroethanol for 20 h. Even the very highly ionizing solvent hexafluoroisopropyl alcohol doesn't lead to solvolysis of **1**. These attempted solvolyses suggest that the cation **3** is relatively unstable and cannot be easily formed under solvolytic conditions. Our findings are also consistent with Moss's report that reaction of methoxybromodiazirine with $AgNO_3$, $AlBr_3$, SbF_5/SO_2, H_2SO_4, $AlCl_3$, AgF, or FSO_3H "failed to provide evidence for diazirinium ions as spectroscopic entities or as chemical intermediates".[6]



In light of the apparent difficulty in generating the diazirinium cation **3**, we reinvestigated the reported isomerization of diethyl maleate to diethyl fumarate catalyzed by **1** where coordination of the cation **3** with the carbonyl oxygen is suggested to initiate the isomerization process.[3] We have found that freshly chromatographed and distilled diazirine **1** does indeed catalyze this interesting isomerization in the nonpolar solvent CCl_4 as reported. However, our lack of evidence for the cation **3** under solvolytic conditions in highly ionizing polar solvents caused us to

(1) (a) Moss, R. A.; Terpinski, J.; Cox, D. P.; Denney, D. Z.; Krogh-Jespersen, K. *J. Am. Chem. Soc.* **1985**, *107*, 2743. (b) Wlostowska, J.; Moss, R. A.; Guo, W.; Chang, M. *Chem. Commun.* **1982**, 432. (c) Cox, D. P.; Moss, R. A.; Terpinski, J. *J. Am. Chem. Soc.* **1983**, *105*, 6513. (d) Moss, R. A.; Kmiecik-Lawrynowicz, G.; Cox, D. P. *Synth. Commun.* **1984**, *14*, 21. (e) Moss, R. A.; Cox, D. P.; Tomioka, H. *Tetrahedron Lett.* **1984**, *25*, 1023. (f) Moss, R. A.; Shen, S.; Hadel, L. M.; Kmiecik-Lawrynowicz, G.; Wlostowska, J.; Krogh-Jespersen, K. *J. Am. Chem. Soc.* **1987**, *109*, 4341.

(2) Liu, M. T. H.; Paike, N. *Tetrahedron Lett.* **1987**, *28*, 3763.

(3) Liu, M. T. H.; Doyle, M. P.; Loh, K.-L.; Anand, S. M. *J. Org. Chem.* **1987**, *52*, 323.

(4) While **1** is recovered essentially unchanged after 1.5 h at room temperature in acetonitrile containing excess $AgNO_3$, under these conditions **1** is slowly converted over a period of 50 days to benzoic acid. The mechanism of this transformation is under investigation.

(5) The reported[2] dissociation constant for **1** in acetonitrile is 2×10^{-5}. If this were correct, then precipitation of AgBr should be instantaneous.

(6) Moss, R. A.; Wlostowska, J.; Guo, W.; Fedorynski, M.; Springer, J. P.; Hirshfield, J. M. *J. Org. Chem.* **1981**, *46*, 5050.

0022-3263/88/1953-4637$01.50/0