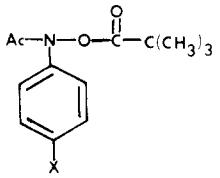
ESR Spin-Trapping Study of the Thermal Decomposition of *N*-Pivaloxyacetanilides in Benzene

Summary: Spin-trapping results favor a mechanism involving homolytic cleavage of the N–O bond for the thermal decomposition in benzene of *N*-pivaloxyacetanilides, which are models for carcinogenic metabolites of aromatic amides.

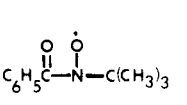
Sir: Sulfuric and carboxylic acid esters of *N*-hydroxy-*N*-arylacetamides are important carcinogenic metabolites derived from polycyclic *N*-arylacetamides including *N*-acetyl-2-aminofluorene.[1,2] Although dismutation reactions of nitroxyl radicals may be involved in the formation of some of these compounds in vivo,[3] it is widely assumed that the sulfate or carboxylate esters react with DNA bases through the intermediacy of an aromatic nitrenium ion formed by heterolysis of the N–O bond.[1,4] However, available data indicate that the bond dissociation energy for the N–O bond in such compounds is in the range of 30–50 kcal/mol.[5] These species should therefore be subject to homolytic N–O bond cleavage to produce radical intermediates. It is of interest to note that many of these sulfate and carboxylate esters attack guanosine at C-8,[1,4] a position more commonly attacked by radical rather than cationic species.[6] We have now obtained spin-trapping[7] results involving ring-substituted *N*-pivaloxyacetanilides **1a–c**[8] which indicate that these compounds do undergo homolytic cleavage in benzene solution.



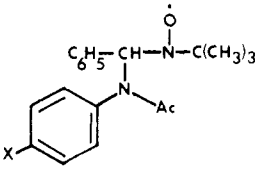
1

	X
1a	CH_3
1b	H
1c	NO_2

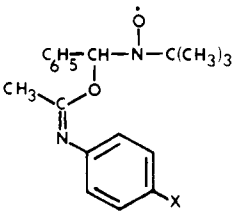
When either 0.02 M solutions of **1a–c** or 0.1 M solutions of *C*-phenyl-*N*-*tert*-butylnitrone (PBN)[9] in degassed benzene were heated separately in an ESR cavity at temperatures as high as 110 °C, no resonance could be detected. However, when **1a–c** and PBN were incubated together at 90 ± 2 °C, signals from three different radical species grew in rapidly over a period of 10–15 min and remained at a steady state for at least several hours.[10] The observed signals and the structures assigned to the corresponding radicals are as follows: (a) a simple triplet (**2**); (b) an 18-line spectrum (**3**); and (c) a triplet of doublets (**4**). (Structural assignments are justified below.) Table



2



3



4

I provides a compilation of hyperfine splitting constants, *g* values, and line widths measured from the spectral data. At higher temperatures (110 °C) and longer incubation times, a fourth signal, a triplet of doublets with hfsc's of 14.6 ± 0.5 G and 2.3 ± 0.2 G, appeared and became a predominant part of the observed spectrum. Spectra obtained at 90 °C could be adequately computer simulated[11] by assuming that only the three previously mentioned radicals contributed to the observed signals. Simulations were performed by using hfsc's, *g* values, and line widths measured directly from the spectra. Only the relative contributions of the three radicals to the observed signal were varied to obtain a fit. Table II summarizes the

(1) Several recent reviews include: Miller, J. A. *Cancer Res.* **1970**, *30*, 559–576. Kriek, E. *Biochim. Biophys. Acta* **1974**, *355*, 177–203. Miller, E. C. *Cancer Res.* **1978**, *38*, 1479–1496. Miller, E. C.; Miller, J. A. *Cancer* **1981**, *47*, 2327–2345.

(2) DeBaun, J. R.; Miller, E. C.; Miller, J. A. *Cancer Res.* **1970**, *30*, 577–595. Weisburger, J. H.; Yamamato, R. S.; Williams, G. M.; Grantham, P. H.; Matsushima, T.; Weisburger, E. K. *Ibid.* **1972**, *32*, 491–500. Kadlubar, F. F.; Miller, J. A.; Miller, E. C. *Ibid.* **1976**, *36*, 2350–2359. King, C. M.; Philipps, B. *J. Biol. Chem.* **1969**, *244*, 6209–6216.

(3) Floyd, R. A. *Can. J. Chem.* **1982**, *60*, 1577–1586 and references therein.

(4) Scribner, J. D.; Miller, J. A.; Miller, E. C. *Cancer Res.* **1970**, *30*, 1570–1579. Scribner, J. D.; Naimy, N. K. *Ibid.* **1973**, *33*, 1159–1164; **1975**, *35*, 1416–1421. Scribner, J. D. *J. Org. Chem.* **1976**, *41*, 3820–3823. Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1981**, *103*, 4281–4291. Toshiharu, O.; Shudo, K.; Okamoto, T. *Tetrahedron Lett.* **1978**, 1983–1986.

(5) The bond dissociation energy of the N–O bond of hydroxylamine has been calculated to be 61 kcal/mol based on heat of formation and vaporization data (Beck, R. A.; Betts, *J. Can. J. Chem.* **1965**, *43*, 2157–2161). This value is comparable to the more accurately determined bond dissociation energies of hydrazine (56 kcal/mol) and hydrogen peroxide (52 kcal/mol) (Kerr, J. A. *Chem. Rev.* **1966**, *66*, 415–500). Substitution of a phenyl group for hydrogen in hydrazine lowers the bond dissociation energy of the N–N bond to 40 kcal/mol, and acyl derivatives of hydrogen peroxide such as acetyl peroxide have even lower bond dissociation energies (ca. 30 kcal/mol) (Kerr, J. A. *Ibid.* **1966**, *66*, 415–500).

(6) (a) Lawley, P. D. In "Chemical Carcinogens"; ACS Monograph No. 173. Searle, C. E., Ed.; American Chemical Society: Washington, DC, 1976; pp 83–244. (b) Magee, P. N.; Montesano, R.; Preussman, R. ref 6a, pp 496–625. (c) Zady, M. F.; Wong, J. L. *J. Am. Chem. Soc.* **1977**, *99*, 5096–5101.

(7) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31–40. Perkins, M. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 1–64.

(8) Details of the synthesis and characterization of **1a–c** are available; see supplementary material section.

(9) PBN was obtained from Aldrich. Identical results were obtained from samples of PBN used without further purification and from samples recrystallized from petroleum ether (mp 75–76 °C). Experiments with nitroso spin traps were also attempted, but under the conditions used for these experiments nitroso compounds rapidly decompose to yield nitroxide radicals, the ESR signals of which obscure any other signals.

(10) All solutions used in this study were prepared under a N_2 atmosphere in a Vacuum Atmospheres HE-43 glovebox equipped with an HE-495 O_2 scrubber. Typically solutions were prepared in the ESR tubes by mixing 12 μL of a 0.50 M CH_3CN solution of the pivalate with 300 μL of benzene containing 0.1 M PBN. The tubes were then sealed with neoprene septum caps before being removed from the glovebox. CH_3CN was purified by stirring over CaH_2 overnight and distilling from P_2O_5 at a high reflux ratio. The first and last 10% of distillate were discarded. The remaining distillate was again stirred over CaH_2 overnight, filtered, and redistilled at a high reflux ratio with the first and last 10% of distillate being discarded. Benzene was washed with concentrated H_2SO_4, followed by 10% NaOH, and finally by deionized water until the washings had a neutral pH. The benzene was then stirred over P_2O_5 overnight, filtered, and distilled at a high reflux ratio. The first and last 10% of distillate were discarded. Both solvents were degassed by a minimum of four freeze–evacuate–thaw cycles before being introduced into the glovebox where all subsequent solutions were prepared. Solutions prepared for ESR were protected from light to minimize the possibility of photochemical reactions. A Varian E-9 ESR equipped with a V-4540 variable temperature controller was used to record the spectra. The temperature controller was calibrated daily. The klystron frequency was monitored by a Hewlett-Packard 5245L frequency counter, and the magnetic field was measured by a Magnion G-502 gaussmeter.

(11) The simulation program was extensively modified by Dr. Frederick T. Greenaway of this department from an original version due to Lefebvere and Maurani: (Lefebvre, R.; Maruani, J. *J. Chem. Phys.* **1965**, *42*, 1480–1496).

0022-3263/84/1949-1142$01.50/0

Table I. Splitting Constants, g Values, and Line Widths (LW) for Radicals Observed in the Spin-Trapping Study of the Decomposition of Pivalates 1a-c

radical	a_N^{NO},[b] G	A_H^{β},[b] G	A_N^{β},[b] G	g[c]	LW,[d] G
2	8.0 ± 0.05			2.0064	0.46
3	14.4 ± 0.1	3.91 ± 0.05	1.54 ± 0.05	2.0056	0.68
4	13.26 ± 0.05	1.68 ± 0.05		2.0060	0.59

[a] All spectra were measured in benzene solution at 90 ± 2 °C. Within experimental error, the substituent X had no effect on the measured parameters for 3 and 4. [b] Reported with estimated limit of error. [c] sd = ±0.0001. [d] Estimated limit of error = ±0.05 G.

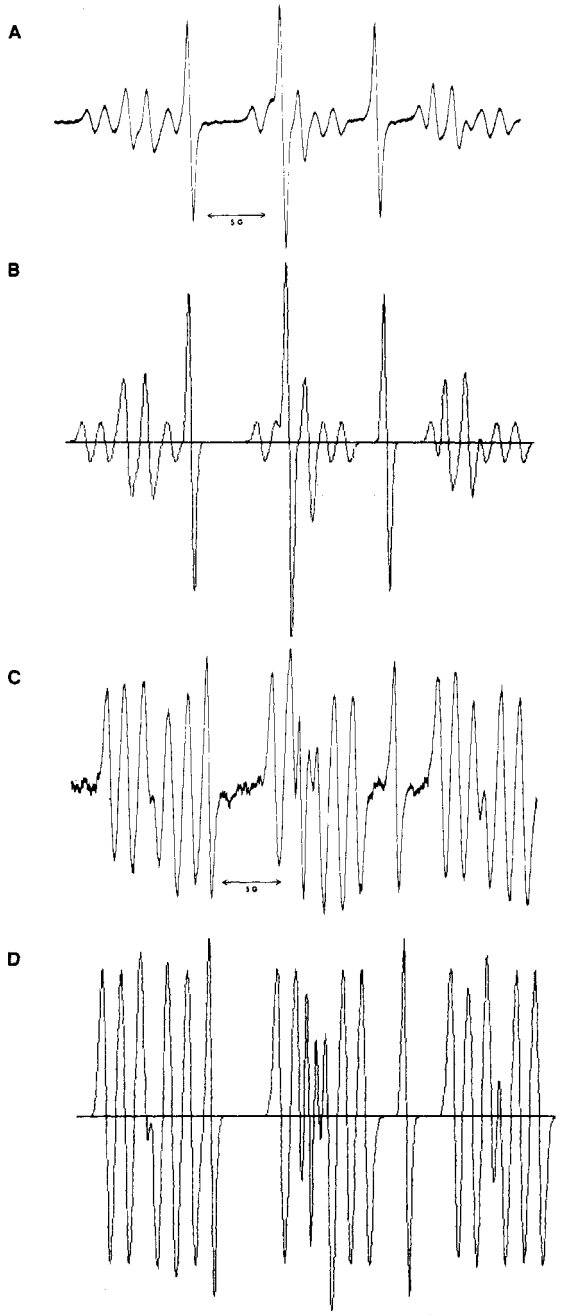


Figure 1. Observed and simulated ESR spectra for compounds **1b** and **1c**: A, observed spectrum for **1b**; B, simulated spectrum for **1b**; C, observed spectrum for **1c**; D, simulated spectrum for **1c**. Vertical scales for observed and simulated spectra are not the same.

contribution of each radical to the observed spectrum as determined by simulations performed for each of the esters **1a–c** at 90 °C. Figure 1 compares observed and simulated spectra for esters **1b** and **1c**.

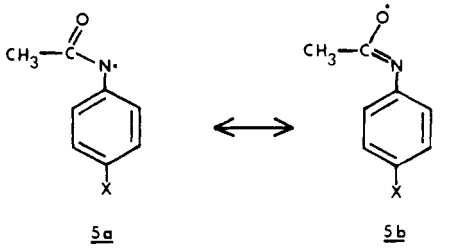
The radical giving rise to the simple triplet has been encountered previously in a number of studies involving PBN and has been identified as benzoyl *tert*-butyl nitroxide (**2**).[12,13] We have identified the radical responsible for the 18-line spectrum as the spin adduct **3**, which results from trapping of the anilido radical **5** by PBN. To our knowledge this is the first published example of the trapping of an amidyl radical by PBN. There have been several reports of spin trapping of amino radicals by nitrones including PBN.[14,15] Coupling constants observed for these species are comparable to those observed for **3**. To confirm the identify of **3**, *N*-bromoacetanilide was prepared[16] and subjected to photolytic decomposition in benzene in the presence of PBN. The ESR spectrum observed at 90 °C was an 18-line spectrum identical with that observed in experiments with **1a–c**. Within experimental error the substituent X has no effect on the g values and coupling constants measured for **3**. This same result has previously been observed in a spin-trapping study of acetanilido radicals with nitrosobenzene.[17]

Table II. Relative Yields of the Three Radical Species as Determined by Simulation

ester	%2[a]	%3[a]	%4[a]
1a	53	10	37
1b	24	44	32
1c	8	86	6

[a] The limit of error for these values is estimated to be ±5% on the basis of the simulation results.

Coupling constants observed for the triplet of doublets are consistent with an acyloxy spin adduct.[12] However, we do not believe that this species could be the pivaloxy adduct of PBN since it can also be generated during the thermal decomposition of *N*-bromoacetanilide in the presence of PBN. We have tentatively identified this species as the *N*-arylacetamido spin adduct **4**. If **4** is correctly identified, then the relative yields of **3** and **4** observed for the three esters (Table II) are consistent with the expected effects of the ring substituents on the relative stabilities of the two canonical forms of the amidyl radical, **5a** and **5b**. We have made no attempt to identify the fourth radical produced at higher temperatures.



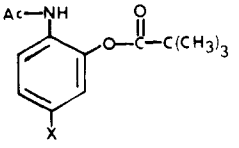
5a 5b

(12) Janzen, E. G.; Blackburn, B. J. *J. Am. Chem. Soc.* **1969**, *91*, 4481–4490.
(13) Forrester, A. R.; Hepburn, S. P. *J. Chem. Soc. C* **1971**, 701–703.
(14) Aurich, H. G.; Suresh, K. D.; Höhlein, P.; Klingelhöfer, H.-G. *Chem. Ber.* **1981**, *114*, 2431–2439.
(15) Ledwith, A.; Russell, P. J. *J. Chem. Soc., Perkin Trans. 2* **1974**, 582–591. Kubow, S.; Dubose, C. M.; Janzen, E. G.; Carlson, J. R.; Bray, T. M. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 168–174.
(16) Slosson, E. E. *Chem. Ber.* **1895**, *28*, 3265–3270.
(17) Chalfont, G. R.; Perkins, M. J. *J. Am. Chem. Soc.* **1967**, *89*, 3054–3055.

The presence of **3** and **4** is most easily explained by homolytic cleavage of the N–O bond of the ester. It has been pointed out previously that an alternative pathway for the production of any radical in the presence of PBN is nucleophilic attack by an anion on the nitrone followed by oxidation of the resulting adduct.[13] This is not a very likely pathway in this case since heterolytic cleavage of the N–O bond would lead to a nitrenium ion, not an amidyl anion.

Benzoyl *tert*-butyl nitroxide (**2**) is generally formed under conditions in which an oxidizing reagent is present.[12,13] There is considerable evidence that nitrenium ions can oxidize such species as I^- and other soft bases.[18] It may be that the presence of **2** indicates that heterolytic N–O bond cleavage is competing with the homolytic process. The relative yield of **2** changes with the substituent X in a manner that is consistent with this hypothesis.

We have not yet characterized the products of the decomposition of **1a–c** in benzene. However in aqueous solution they decompose predominantly to give *O*-pivaloxyacetanilides **6**. *N*-(benzoyloxy)benzanilide decomposes



6

when heated in decalin, nitrobenzene, and DMF to yield the analogous *O*-(benzoyloxy)benzanilide and other products such as benzanilide and *N*-benzoyl-4-aminobiphenyl, which are best explained by radical reaction processes.[19]

Our results provide support for a mechanism involving homolytic N–O bond cleavage for the recently reported cross-linking of DNA and protein mediated by *N*-acetoxy-*N*-acetyl-2-aminofluorene, a reaction that is sensitive to the presence of radical traps.[20] Other reactions of the carcinogenic esters, including attack at C-8 of guanosine, may proceed via radical pathways also.

We are expanding our studies of the radical reactions of these esters with an emphasis on exploring the viability of such reactions in more polar solvents.

Acknowledgment. We are grateful for the assistance of Bruce Huberman, Lee Pollack, and John Dionis who aided in the syntheses of compounds **1a–c**. We are also thankful to Dr. Fred Greenaway of this department who provided us with the simulation program and many helpful suggestions. B.A.B. would like to thank the General Electric Foundation for a Summer Research Fellowship. This work was supported by grants from the Petroleum Research Fund, administered by the American Chemical Society, and the American Cancer Society (BC-348).

Registry No. **1a**, 88867-64-5; **1b**, 88867-65-6; **1c**, 88887-33-6; **2**, 35822-90-3; **3** (X = Me), 88887-34-7; **3** (X = H), 88867-66-7; **3** (X = NO_2), 88867-67-8; **4** (X = Me), 88887-35-8; **4** (X = H), 88867-68-9; **4** (X = NO_2), 88867-69-0; *N*-phenylhydroxylamine, 100-65-2; *N*-hydroxyacetanilide, 1795-83-1; pivaloyl chloride, 3282-30-2; *N*-(*p*-tolyl)hydroxylamine, 623-10-9; *N*-hydroxy-*p*-acetotoluide, 27451-21-4; *O*-pivaloyl-*N*-(*p*-nitrophenyl)-hydroxylamine, 88867-70-3; *N*-(*p*-nitrophenyl)hydroxylamine, 16169-16-7.

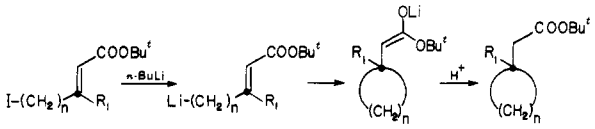
Supplementary Material Available: Details of the syntheses and characterization of **1a–c** (4 pages). Ordering information is given on any current masthead page.

Michael Novak,* Brenda A. Brodeur
Department of Chemistry, Clark University
Worcester, Massachusetts 01610
Received November 2, 1983

Metal–Halogen Exchange-Initiated Intramolecular Conjugate Addition Reactions of Unsaturated Esters

Summary: Intramolecular conjugate addition reactions of internal unstabilized nucleophilic centers formed through rapid lithium–halogen exchange reactions have been used to generate carbocycles from ω-iodo-α,β-unsaturated esters.

Sir: There has been much recent interest in carbocycle synthesis via both intramolecular radical cyclizations[1] and intramolecular Michael addition reactions of stabilized carbanionic centers.[2] We now describe cyclization reactions of ω-iodo-α,β-unsaturated esters **1**, which presumably proceed through highly unstabilized nucleophilic intermediates **2** formed by rapid lithium–halogen exchange. While there are several examples of extremely rapid exchange reactions with cyclopropyl[3] and aryl halides,[4] little is known about the relative rates of such reactions at saturated centers.[5] We have found that such exchange reactions are in some cases rapid enough to permit the formation of unstabilized nucleophilic centers in the presence of Michael acceptors with which they may subsequently undergo direct intramolecular addition reactions.[6] Results of cyclizations of the type depicted in eq 1 are shown in Table I.



1 **2** **3** **4**

(1) Danishefsky, S.; Chackalamannil, S.; Uang, B.-J. *J. Org. Chem.* **1982**, *47*, 2231. Stork, G.; Mook, R., Jr.; Biller, S. A.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **1983**, *105*, 3741. Stork, G.; Mook, R., Jr. *Ibid.* **1983**, *105*, 3720. Stork, G.; Baine, N. H. *Ibid.* **1982**, *104*, 2321. Marinovic, N. N.; Ramanathan, H. *Tetrahedron Lett.* **1983**, *24*, 1871. Chuang, C.-P.; Hart, D. J. *J. Org. Chem.* **1983**, *48*, 1782. Corey, E. J.; Pyne, S. G. *Tetrahedron Lett.* **1983**, *24*, 2821.

(2) For recent examples and leading references, see: Majetich, G.; Desmond, R.; Casares, A. M. *Tetrahedron Lett.* **1983**, *24*, 1913. Stork, G.; Winkler, J. D.; Saccomano, N. A. *Ibid.* **1983**, *24*, 465.

(3) Stein, C. A.; Morton, T. H. *Tetrahedron Lett.* **1973**, 4934.

(4) Boatman, R. J.; Whitlock, B. J.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 4822.

(5) Applequist, D. E.; O'Brien, D. F. *J. Am. Chem. Soc.* **1963**, *85*, 743. Jones, R. G.; Gilman, H. *Org. React. (N.Y.)* **1951**, *6*, 339. For a recent report on the primacy of the metal–halogen exchange reaction of 3,3-difluoro-3-bromopropene with *n*-BuLi in the presence of carbonyl-containing compounds, see: Seyferth, D.; Simon, R. M.; Sepelak, D. J.; Klein, H. A. *J. Am. Chem. Soc.* **1983**, *105*, 4634.

(6) 2,2-Dimethyl-6-iodohexanenitrile reportedly gives 2,2-dimethylcyclohexane in unspecified yield upon treatment with *n*-BuLi through a pathway thought to involve metal–halogen exchange followed by an intramolecular 1,2-addition reaction: Larcheueque, M.; Debal, A.; Cuvigny, T. *J. Organometal. Chem.* **1975**, *87*, 25. The recently reported cyclization of α,ω-diiodides with *t*-BuLi is likely an example of an exchange-initiated alkylation reaction: Bailey, W. F.; Gagnier, R. P. *Tetrahedron Lett.* **1982**, *23*, 5123.

(18) Parham, J. C.; Templeton, M. A.; Teller, M. N. *J. Org. Chem.* **1978**, *43*, 2325–2330. Parham, J. C.; Templeton, M. A. *Cancer Res.* **1980**, *40*, 1475–1481; *J. Org. Chem.* **1982**, *47*, 652–657. Novak, M.; Pelecanou, M.; Roy, A. K.; Andronico, A. F.; Plourde, F. M.; Olefirowicz, T. M. *J. Am. Chem. Soc.*, submitted for publication.

(19) Horner, L.; Steppan, H. *Liebigs Ann. Chem.* **1957**, *606*, 24–47.

(20) Rayshell, M.; Ross, J.; Werbin, H. *Carcinogen.—Compr. Surv.* **1983**, *4*, 501–507.

0022-3263/84/1949-1144$01.50/0