Isolation of (+)-(1*R*,2*S*,3*R*)-*cis*,*trans*-2,3-Dideuterio-*trans*-2-spiro[cyclopropane-1,1'-indene]carboxylic Acid ((+)-22). The trans acid (+)-**22** was separated from the mixture of (+)-**22** and (+)-**19** by fractional crystallization of the respective quinine salts. The 86:14 mixture of acids (+)-**22** and (+)-**19** (1.10 g, 5.85 mmol) was dissolved in a hot mixture of absolute ethanol (6 mL) and hexanes (6 mL). This solution was added to a hot solution of quinine (1.85 g, 5.74 mmol) in ethanol (12 mL). The following day, filtration gave 2.22 g of white crystalline quinine salt. This salt was dissolved in a minimum volume of hot absolute ethanol (about 20 mL), and hexanes (5 mL) were then added before the solution was allowed to cool. Filtration of the solution furnished 1.71 g of crystalline salt. After two more recrystallizations there remained 1.22 g of salt having $[\alpha]_{546}$ +257.2° (ethanol). The mother liquors were combined and fractionally crystallized in a similar manner to yield only 0.22 g of material of comparable isomeric purity (assessed by NMR after hydrolysis of a small sample). The remainder of the salt, when forced to yield crystalline material, gave a eutectic mixture of the two quinine salts in a 3:2 ratio favoring the salt of the trans isomer, (+)-**22**.

The two samples of 1.22 g and 0.22 g were combined and hydrolyzed in 10% aqueous hydrochloric acid. The solution was extracted with ether (5 × 25 mL), and the combined extracts were washed (10% aq HCl, H_2O, brine), dried ($MgSO_4$), filtered and concentrated to yield 548.5 mg of white crystalline material, mp 164–166°C; NMR δ 1.97 (1 H, s), 6.59 (1 H, d, J = 6), 6.93 (1 H, d, J = 6), 7.02–7.44 (4 H, m), 11.58 (1 H, broad s).

(1*R*,2*S*,3*R*)-*cis*,*trans*-2,3-Dideuterio-*trans*-2-(hydroxymethyl)spiro[cyclopropane-1,1'-indene] (17). Reduction of carboxylic acid (+)-**22** (548 mg, 2.91 mmol) was accomplished by using lithium aluminum hydride in an ether solution as described previously. This procedure yielded 484 mg (97%) of alcohol as a clear colorless oil; NMR δ 1.56 (1 H, broad s), 1.76 (1 H, s), 3.81 (2 H, AB, $\Delta\nu$ = 0.37, J = 12), 6.36 (1 H, d, J = 6), 6.98 (1 H, d, J = 6), 6.85–7.50 (4 H, m).

(+)-(1*R*,2*S*,3*R*)-*cis*,*trans*-2,3-Dideuterio-*trans*-2-(methoxymethyl)-spiro[cyclopropane-1,1'-indene] ((+)-2-c). Alkylation of the alcohol prepared immediately above (484 mg, 2.79 mmol) using the procedure previously described for alkylation of the unlabeled alcohol gave 544 mg of an orange oil. An analytical sample was purified by VPC (column C, 165 °C); $[\alpha]_{546}$ 269.0° ($CHCl_3$); NMR δ 1.74 (1 H, s), 3.34 (1 H, s), 3.61 (2 H, AB, $\Delta\nu$ = 0.10 ppm, J = 9), 6.35 (1 H, d, J = 6), 6.93 (1 H, d, J = 6), 7.02–7.48 (4 H, m); IR ($CHCl_3$) 3050, 3000, 2930, 2890, 2825, 1457, 1378, 1168, 1103, 994, 947, 896, 820; MS, m/z (rel intensity) 188 (M^+, s), 154 (6), 153 (5), 142 (9), 130 (5), 129 (100), 116 (15).

This material was purified by VPC (column C, 165°C) before thermolysis. Careful analysis of the purified material by NMR showed that it contained approximately 0.4% of cis isomer **1-c**. This was apparently derived from incomplete separation of the trans and cis acids (+)-**22** and (+)-**19**, and was therefore assigned the (+)-**1-c** configuration in order to account for its presence explicitly in kinetic analyses.

Methyl (−)-1,1,2-Cyclopropanetricarboxylate ((−)-24). To a solution of partially resolved trans acid (−)-**7** (168 mg, 0.89 mmol, $[\alpha]_{546}$ −238.4° ($CHCl_3$)) in a mixture of acetonitrile (4 mL), carbon tetrachloride (4 mL), and water (8 mL) was added 3.8 g (17.8 mmol) of sodium periodate (sodium metaperiodate) and a catalytic amount (~5 mg) of $RuCl_3(H_2O)_{1-3}$.[27]

The solution was stirred vigorously for 72 h at room temperature before acetone (50 mL) was added, and the resulting solution was filtered and concentrated to dryness. The 325 mg of white solid obtained was then dissolved in 15 mL of methanol and treated with an ethereal solution of diazomethane until a yellow color persisted in solution. During this addition some material precipitated from solution and was removed by filtration after the excess diazomethane had been quenched through dropwise addition of acetic acid. The solution was concentrated to a volume of 3 mL by distillation of solvent through a 10-cm Vigreux column. The product was purified by VPC (column C, 135 °C) to give 40 mg of (−)-**24**, $[\alpha]_{546}$ −37.9° ($CHCl_3$). The spectral features of this material were identical with those previously reported.[28]

Sealed Tube Kinetics. The pyrolysis ampules were prepared from 7-mm borosilicate glass tubing. The tubes were soaked in an ammonium hydroxide/EDTA solution at 60 °C for at least 24 h and then rinsed well with distilled water and dried in an oven at 130 °C for 24 h.

Methyl ethers (−)-**1-c** and (+)-**2-c** were purified by VPC (column C, 165 °C), and samples of 40–100 mg were diluted with toluene to a volume of about 0.75 mL and sealed under vacuum (10^{-5} torr) in the pyrolysis ampules. The sealed ampules were typically 8 cm long.

The ampules were attached to a wire cage and submerged in a molten salt bath heated to 198.9 °C, as determined with a Hewlett-Packard 2802 A platinum resistance thermometer. The temperature of the bath decreased to 198.6 °C upon immersion of a tube, but recovered to a temperature of 198.9 °C within 2 min. Following a period in the kinetic bath, a pyrolysis tube was immediately cooled with tap water. The ratio of the four degenerate cis isomers to the set of four degenerate trans isomers was determined by VPC using column E with an oven temperature of 168 °C. These conditions produced retention times of approximately 140 and 160 min for the cis and trans isomers, respectively. The two isomer sets were then preparatively separated by using VPC (column D, 165 °C; retention times of 108 and 129 min, respectively). The set of four cis isomers (**1**) was first analyzed by polarimetry. Optical rotations were measured at a wavelength of 546 nm on samples of 11–20 mg dissolved in 4 mL of chloroform. The solvent was then removed, and the samples were redissolved in chloroform-*d* for analysis by NMR at 360 MHz.

The vapor-phase chromatographically homogeneous set of four degenerate trans isomers was first dissolved in chloroform-*d* for preliminary analysis by NMR (360 MHz). This solvent was then removed and replaced by benzene-d_6 before further analysis by NMR in the presence of an optically active shift reagent ($Eu(hfc)_3$).

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Supplementary Material Available: Ten NMR and $Eu(hfc)_3$ NMR spectra for representative substrates and thermolysis product mixtures (5 pages). Ordering information is given on any current masthead page.

Carbonyl Oxide Chemistry. The NIH Shift

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Abstract: Benzophenone oxide has been shown to oxidize 1-chloronaphthalene and *p*-xylene with accompanying NIH shift of chlorine and methyl, respectively. Similar oxidation of toluene leads to a mixture of *o*-, *p*-, and *m*-cresols while *N*-acetyl-L-phenylalanine ethyl ester is oxidized to the corresponding tyrosine derivative. The results are discussed in terms of their relationship to the "activation" of polycyclic aromatic hydrocarbons in polluted atmospheres and the possible production of mutagens/carcinogens.

Criegee was the first to suggest that carbonyl oxides are important intermediates in the ozonolysis reaction.[1] The experimental evidence[2] is overwhelmingly supportive of this suggestion. Interestingly enough there has been only one report[3] of the physical characterization of a carbonyl oxide. In addition, there are a few reports[4-6] describing the characterization by physical methods of

† Taken in part from a dissertation submitted by Shailendra Kumar to the faculty of the University of Missouri-St. Louis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(1) Criegee, R. *Rec. Chem. Progr.* **1957**, *18*, 111.
(2) Bailey, P. S. "Ozonation in Organic Chemistry"; Academic Press: New York, 1978; Vol. I.
(3) Chapman, O. L.; Hess, T. C. *J. Org. Chem.* **1979**, *44*, 962 and ref 1 therein.

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the carbonyl oxide isomer, dioxirane.

Carbonyl oxides produced via ozonolysis have been shown to give ozonides, di- and triperoxides, oligomers, esters, and, in reactive solvents, alkoxy and other hydroperoxides.[2] The usual conditions of the ozonolysis reaction have made it difficult to study other potential chemistry of carbonyl oxides, however. These interesting intermediates can also be obtained via oxygenation of diazo compounds,[7-27] and we have shown[12-16] that the use of singlet oxygen makes this method a convenient carbonyl oxide source. Carbonyl oxides obtained in this manner have been trapped by aldehydes to give ozonides[10-12] and also by nucleophilic solvents such as methanol.[26]

The suggestion by Hamilton[7,28] that oxidation reactions catalyzed by those monooxygenase enzymes (MOX) requiring cofactor flavin may proceed via oxenoid intermediates, such as a carbonyl oxide, has served to stimulate further interest in carbonyl oxide chemistry. Thus, carbonyl oxides, produced by the oxygenation of various diazo compounds, have been shown to carry out one-oxygen-atom oxidations of alkanes,[7] alkenes,[14] aromatic compounds,[13,15-18,29] sulfides,[20,21,30,31] and sulfoxides[20,21,30-32] and to bring about oxidative decarboxylations of α-keto carboxylic acids.[24]

It is now generally recognized[33,34] that carcinogenic polycyclic aromatic hydrocarbons (PAH) require metabolic activation prior to becoming bound to biological macromolecules. The necessary activation is brought about by monooxygenase enzymes.[35,36] Included among the PAH metabolites are arene oxides[37-39] and diol epoxides.[40] In the case of benzo[*a*]pyrene certain diol epoxides are recognized as the most mutagenic[41-43] and carcinogenic.[44,45]

Since some polluted atmospheres contain PAH, olefins, and ozone we have suggested[46] that carbonyl oxides, produced from ozone–olefin reactions in these atmospheres, could react with, i.e., "activate", atmospheric PAH and produce arene oxides. We have reported[13] that oxidation of naphthalene with the carbonyl oxide, benzophenone oxide, leads to a mixture of α- and β-naphthols. The naphthol distribution is similar to that obtained[47] by rearrangement of naphthalene 1,2-oxide. With use of a series of substituted benzophenone oxides and a Hammett $\rho\sigma$ relationship this naphthalene oxidation has been shown[16] to be electrophilic in nature. While the presumed arene oxide intermediate could not be isolated in the case of naphthalene, oxidation of phenanthrene under the same conditions led to the isolation[46] of the K-region oxide, phenanthrene 9,10-oxide. We have also been able to show[15,16] that some aryl carbonyl oxides can transfer the O atom intramolecularly. We recently described[29] a case of intramolecular O-atom transfer from a carbonyl oxide to give a K-region oxide. Indeed, in this case, the carbonyl oxide was produced via ozonolysis of an olefin as well as by the non-ozone source. The results of the work to date on carbonyl oxide–aromatic substrate reactions add support to our speculation regarding nonenzymatic activation of PAH in polluted atmospheres.

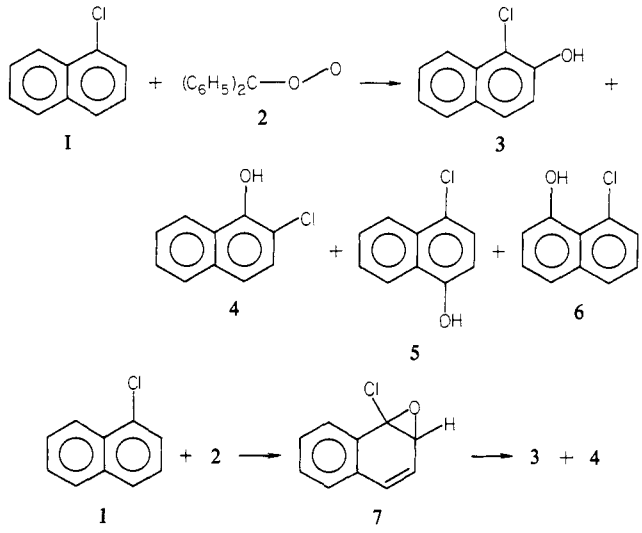
In a very significant contribution to aromatic substrate carbonyl oxide chemistry Jerina et al.[17,18] have shown that fluorenone oxide is able to hydroxylate 4-D-anisole with accompanying NIH shift[48,49] of deuterium. In this paper we describe further examples of oxidation of aromatic substrates by carbonyl oxides including examples in which NIH shift by chlorine and methyl has occurred.

Results and Discussion

Photosensitized oxidation of diphenyldiazomethane in the presence of 1-chloronaphthalene (**1**) gave a product mixture from which four isomeric chloronaphthols could be isolated. The chloronaphthols are believed to be produced by oxidation of **1** with benzophenone oxide (**2**), the carbonyl oxide produced by singlet oxygen oxidation[12-16] of the diazo compound. The products were identified as 1-chloro-2-naphthol (**3**), 2-chloro-1-naphthol (**4**), 4-chloro-1-naphthol (**5**), and 8-chloro-1-naphthol (**6**). Product **5** was obtained in 4.3% yield and each of the others was found in 0.8% yield. Control reactions indicated that both diphenyldiazomethane and singlet oxygen are required for the observed oxidation of **1**.

While the details of the oxygen atom transfer reaction are unclear the observation of the NIH shift product, **4**, is taken as a good indication of the intermediacy of the arene oxide, **7**. Such 1,2 shifts in aromatic substrates are generally interpreted[50] as

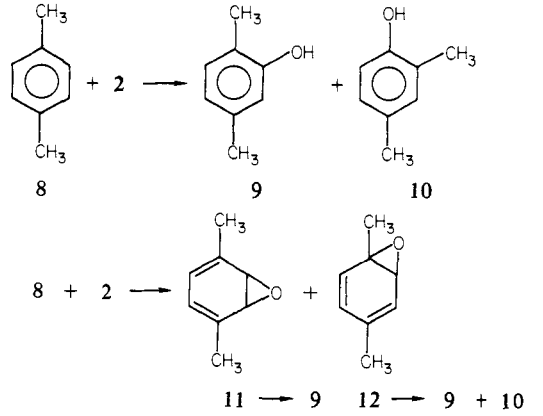
(4) Lovas, F. J.; Suenram, R. D. *Chem. Phys. Lett.* **1977**, *51* 453.
(5) Martinez, R. I.; Huie, R. E.; Herron, J. T. *Chem. Phys. Lett.* **1977**, *51*, 457.
(6) Suenram, R. D.; Lovas, F. J. *J. Am. Chem. Soc.* **1978**, *100*, 5117.
(7) Hamilton, G. A.; Giacin, J. R. *J. Am. Chem. Soc.* **1966**, *88*, 1584.
(8) Kirmse, W.; Horner, L.; Hoffman, H. *Liebigs Ann. Chem.* **1966**, *31*, 419.
(9) Bartlett, P. D.; Traylor, T. G. *J. Am. Chem. Soc.* **1962**, *84*, 3408.
(10) Murray, R. W.; Suzui, A. *J. Am. Chem. Soc.* **1971**, *93*, 4963.
(11) Murray, R. W.; Suzui, A. *J. Am. Chem. Soc.* **1973**, *95*, 3343.
(12) Higley, D. P.; Murray, R. W. *J. Am. Chem. Soc.* **1974**, *96*, 3330.
(13) Chaudhary, S. K.; Hoyt, R. A.; Murray, R. W. *Tetrahedron Lett.* **1976**, 4235.
(14) Hinrichs, T. A.; Ramachandran, V.; Murray, R. W. *J. Am. Chem. Soc.* **1979**, *101*, 1282.
(15) Kumar, S.; Murray, R. W. *Tetrahedron Lett.* **1980**, 4781.
(16) Agarwal, S. K.; Murray, R. W. *Photochem. Photobiol.* **1982**, *35*, 31.
(17) Daly, J. W.; Jerina, D. M.; Witkop, B. *Experimentia* **1972**, 28, 1129.
(18) Jerina, D. M.; Body, D. R.; Daly, J. W. *Tetrahedron Lett.* **1970**, 457.
(19) Ryang, H. S.; Foote, C. S. *J. Am. Chem. Soc.* **1980**, *102*, 2129.
(20) Ando, W.; Kabe, Y.; Miyazaki, H. *Photochem. Photobiol.* **1980**, *31*, 191.
(21) Ando, W.; Kohmoto, S.; Miyazaki, H.; Nishizawa, K.; Tsumaki, H. *Photochem. Photobiol.* **1979**, *30*, 81.
(22) Sekiguchi, A.; Kabe, Y.; Ando, W. *J. Org. Chem.* **1982**, *47*, 2900.
(23) Ando, W.; Miyazaki, H.; Ueno, K.; Nakanishi, H.; Sakurai, T.; Kobayashi, K. *J. Am. Chem. Soc.* **1981**, *103*, 4949.
(24) Ando, W.; Miyazaki, H.; Akasaka, T. *Tetrahedron Lett.* **1982**, 1197.
(25) Ando, W.; Miyazaki, H.; Ito, K.; Auchi, D. *Tetrahedron Lett.* **1982**, 555.
(26) Ryang, H. S.; Foote, C. S. *J. Am. Chem. Soc.* **1981**, *103*, 4951.
(27) Sekiguchi, A.; Kabe, Y.; Ando, W. *J. Chem. Soc., Chem. Commun.* **1979**, 233.
(28) Hamilton, G. A. In "Progress in Bioorganic Chemistry"; Kaiser, E. T., Kezdy, F. J. Eds.; Wiley: New York, 1971; Vol. 1, 83.
(29) Murray, R. W.; Banavali, R. *Tetrahedron Lett.* **1983**, *24* 2327.
(30) Ando, W.; Kohmoto, S.; Nishizawa, K. *J. Chem. Soc., Chem. Commun.* **1978**, 894.
(31) Ando, W.; Miyazaki, H.; Kohmoto, S. *Tetrahedron Lett.* **1979**, 1317.
(32) Sawaki, Y.; Kato, h.; Ogata, *J. Am. Chem. Soc.* **1981**, *103* 3832.
(33) Harvey, R. G. *Acc. Chem. Res.* **1981**, *14*, 218.
(34) Tsang, W. S.; Griffin, G. W. "Metabolic Activation of Polynuclear Aromatic Hydrocarbons"; Pergamon: New York, 1979.
(35) Goshman, L. M.; Heidelberger, C. *Cancer Res.* **1967**, *27*, 1678.
(36) Grover, P. L.; Sims, P. *Biochem. J.* **1968**, *110* 159.
(37) Sims, P.; Grover, P. L. *Adv. Cancer Res.* **1974**, *20*, 165.
(38) Daly, J. W.; Jerina, D. M.; Witkop, B. *Experientia* **1972**, *28*, 1129.
(39) Yang, S. K.; Roller, P. P.; Fu, P. P.; Harvey, R. G.; Gelboin, H. V. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 1176.
(40) Jims, P.; Grover, P. L.; Swaisland, A.; Pal, K.; Hewer, A. *Nature (London)* **1974**, *252* 326.
(41) Newbold, R. F.; Brookes, P. *Nature (London)* **1976**, *261* 53.
(42) Huberman, E.; Sachs, L.; Yang, S. K.; Gelboin, H. V. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 607.
(43) Wood, A. W.; Wislocki, P. C.; Chang, R. L.; Levin, W.; Lu, A. Y.; Yagi, H.; Hernandez, O.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1976**, *36*, 3358.
(44) Marquardt, H.; Baker, S. *Cancer Lett.* **1977**, *3*, 31.
(45) Slaga, T. J.; Bracken, W. M.; Viaje, A.; Levin, W.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1977**, *37*, 4130.
(46) Murray, R. W.; Kumar, S. In "Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry"; Cooke, M., Dennis, A. J., Fisher, G. L., Eds.; Battelle, 1982; p 575.
(47) Jerina, D. M.; Daly, J. W.; Witkop, B.; Saltzman-Nirenberg, P.; Udenfriend, S. *Biochemistry* **1970**, *9*, 147.
(48) Guroff, G.; Reifsnyder, C. A.; Daly, J. W. *Biochem. Biophys. Res. Commun.* **1966**, *24*, 720.
(49) Guroff, G.; Kondo, K.; Daly, J. W. *Biochem. Biophys. Res. Commun.* **1966**, *25*, 622.



indicating arene oxide intermediates in the enzyme-catalyzed oxidations. One of the earliest observations of the NIH shift was the production of *m*-chlorotyrosine in the hydroxylation of *p*-chlorophenylalanine by bacterial phenylalanine hydroxylase or liver phenylalanine hydrolyase.[49] There have been additional reports[51-53] of such chlorine shifts occurring in the metabolism of chlorine-containing substrates.

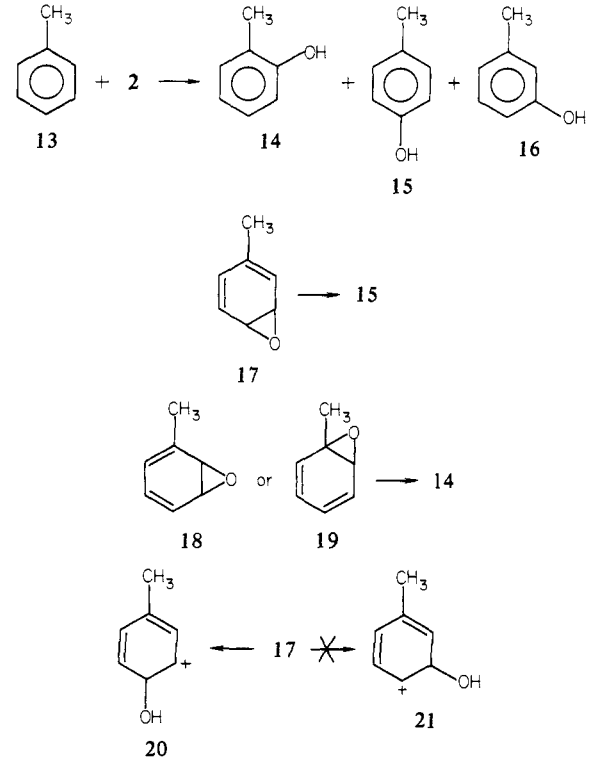
All of our work on the oxidation of aromatic compounds to this point has involved systems containing more than one ring. As mentioned earlier Jerina et al.[17,18] have shown that the carbonyl oxide, fluorenone oxide, can oxidize the single ring aromatic, 4-D-anisole, with accompanying NIH shift of deuterium. We were encouraged therefore to study the oxidation of a number of mono-ring aromatics. The choice of *p*-xylene (**8**) as a substrate reduces the number of potential oxidation products.

Oxidation of **8** by carbonyl oxide **2** in acetonitrile solvent led to the formation of two products, 2,5-xylol (**9**) and 2,4-xylol (**10**), in a 6:1 ratio and in 2.5% total yield. Formation of xylol **10** requires a 1,2 migration of a methyl group. Oxenoid oxidation of **8** by **2** can proceed via oxiranes **11** or **12**. Either intermediate can lead to the formation of **9**; however, only **12** can give the methyl-shifted product **10**.



Enzymatic oxidation of **8** in vitro or in vivo produces exclusively **9**.[54-56] Absence of the methyl-migrated product **10** in these oxidations rules out the intermediacy of arene oxide **12** since it has been shown separately[54] that **12** isomerizes to both **9** and **10**. On the other hand, the enzymatic oxidation of 4-methylphenylalanine by phenylalanine hydroxylase leads to a mixture of 3-hydroxy-4-methylphenylalanine and 4-hydroxy-3-methylphenylalanine.[57] The latter is the product expected from an NIH shift in an intermediate arene oxide. Furthermore, the arene oxide would have to be located at the site of methyl substitution.

Next toluene (**13**), taken as representative of monoalkylbenzenes, was subjected to oxidation by carbonyl oxide **2** in methylene chloride solution. A combination of TLC and GLC workups disclosed the presence of all three possible cresol products, *o*-cresol (**14**) (1.3%), *p*-cresol (**15**) (0.8%), and *m*-cresol (**16**) (0.9%). Oxidation of **13** via the arene oxide route can, in theory, lead to the formation of three possible arene oxides, **17**, **18**, and **19**. Independent synthesis and subsequent rearrangement of the oxides indicates[56] that oxide **17** gives exclusively *p*-cresol (**15**), while acid-catalyzed rearrangement of oxides **18** and **19** leads in both cases to *o*-cresol (**14**); i.e., under these conditions neither **17** nor **18** gave the theoretically possible cresol isomer **16**.



Oxidation of **13** with use of rat liver microsomes leads exclusively[56] to **14** and **15**. The failure to observe *m*-cresol (**16**) from rearrangement of oxides **17** or **18** is explained[56] on the basis of additional stabilization of cationoid intermediates, e.g., **20**, in which the methyl group can interact with the positive charge as opposed to **21** in which such additional stabilization is absent.

Our previous results, including isolation of phenanthrene oxide[46] and observation of the NIH shift in the oxidation of **1** and **8**, suggest that the carbonyl oxide oxidation of **13** probably proceeds via arene oxide intermediates, i.e., **17**, **18**, and **19**. The fact that we obtain the *m*-cresol isomer (**16**) while the oxides **17** and **18** had previously been shown not to give **16** can perhaps be understood by observing that the conditions for rearrangement of the oxides are different than the acid-catalyzed ones used previously.

We next studied the oxidation of a substrate more closely related to biological substrates. The phenylalanine derivative, *N*-acetyl-L-phenylalanine ethyl ester (**22**), was subjected to oxidation by carbonyl oxide **2** in acetonitrile solution. With use of reaction workups involving column chromatography–preparative TLC or

(50) Reed, D. J.; Vimmerstedt, J.; Jerina, D. M.; Daly, J. W. *Arch* Biochem. *Biophys.* **1973**, *154*, 642.
(51) Faulkner, J. K.; Woodcock, D. *J. Chem. Soc.* **1965**, 1187.
(52) Thomas, E. W.; Loughman, B. C.; Powell, R. G. *Nature* (*London*) **1964**, *204*, 884.
(53) Safe, S.; Jones, D. *J. Chem. Soc., Perkin Trans. 1* **1976**, 357.
(54) Bakke, O. M.; Scheline, R. R. *Toxicol. Appl. Pharmacol.* **1970**, *16*, 691.
(55) Jerina, D. M.; Kaubisch, N.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2545.
(56) Kaubisch, N.; Daly, J. W.; Jerina, D. M. *Biochemistry* **1972**, *11*, 3080.
(57) Daly, J. W.; Guroff, G. *Arch. Biochem. Biophys.* **1968**, *125*, 136.

HPLC–preparative TLC the reaction was found to give a 1% yield of *N*-acetyl-L-tyrosine ethyl ester (**23**). No attempt was made to isolate or identify any other reaction products. By analogy to the previous cases of aromatic substrate oxidation we believe that the oxidation of **22** to **23** involves an arene oxide intermediate. The biosynthesis of tyrosine involves the hydroxylation of phenylalanine by phenylalanine hydroxylase. It was during a study of this reaction, using 4-T-phenylalanine, that the NIH shift was discovered[48,49] and attention focused on arene oxide intermediates.

Summary and Significance

The use of the singlet oxygen–diazo compound method has permitted us to demonstrate several additional examples of oxidation of aromatic substrates by carbonyl oxides. The observation of NIH shift of methyl and chlorine as well as product composition leads us to conclude that these oxidations proceed through arene oxide intermediates. These results as well as those reported earlier by us[13,15,16,29] and Jerina et al.[17,18] lend support to our speculation that similar reactions may be occurring in polluted atmospheres. Such atmospheres frequently contain ozone, olefins, and PAH. Reaction of ozone with atmospheric olefins could produce carbonyl oxides which in turn could oxidize PAH to arene oxides, possibly including mutagenic varieties. We have recently shown[29] that an aromatic-substituted olefin absorbed on silica gel reacts with ozone in a gas stream to give the K-region oxide of the aromatic group. This reaction could serve as a model for similar reactions on particulate surfaces in polluted atmospheres. It is significant that atmospheric particulate matter frequently displays[58-60] a carcinogenic activity which is greater than that associated with the known PAH content, i.e., there is indication of non-enzymatic "activation" processes occurring in such atmospheres.

Experimental Section

Instrumentation. Infrared spectra were recorded on Perkin-Elmer Model 137 and 337 infrared spectrophotometers with matched 0.1-mm NaCl solution cells obtained from Wilmad Glass Co., Buena, NJ. The spectra were calibrated with the 1601-cm^{-1} band of polystyrene film. Nuclear magnetic resonance spectra were obtained with a Varian T-60 NMR spectrometer. Deuterated chloroform containing 1% tetramethylsilane (Aldrich) was used as a solvent. Mass spectra were run on an Associated Electronics Industries MS 1201-B mass spectrometer operated at 70-eV ionizing voltage. Gas chromatography was performed on Varian Aerograph Model A-700 and A-705 gas chromatographs. High performance liquid chromatography was carried out on a Varian Model 5020 gradient liquid chromatograph with a variable wavelength Varian Vari-chrom detector set at 254 nm. Fractions from column chromatography were collected by using a Warner-Chilcott Model 1205 automatic fraction collector. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and on a Dynamics optics hot-stage microscope.

Chromatography. Preparative thin layer chromatography plates (20 × 20 cm) precoated with 0.25- and 1.0-mm thicknesses of silica gel with fluorescent indicator UV-254 were purchased from Brinkmann Instruments, Inc., Des Plaines, IL. Analytical thin layer chromatography plastic plates precoated with a 0.1-mm thickness of silica gel with fluorescent indicator UV-254 were obtained from Eastman Kodak Co., Rochester, NY. Alumina (activity 1, 80–200 mesh) for column chromatography was purchased from Fisher Scientific Co., Fairlawn, NJ, and was converted to activity 3 by shaking it with 6% water by weight. Silica gel (80-100 mesh) for column chromatography was purchased from Fisher Scientific Co., Fairlawn, NJ.

Gas chromatography columns 15% SE-30 on Chromosorb W (30–60 mesh), $^3/_4$ in. × 6 ft; 3% tricresylphosphate on gas chrom-Q (100–200 mesh), $^1/_4$ in. × 15 ft; and 6% tricresylphosphate on gas chrom-Q (100–200 mesh), $^3/_8$ in. × 20 ft, were purchased from Varian Aerograph, Palo Alto, CA. HPLC columns [cyano silica, 8 mm × 50 cm (cyano-10)] and reverse phase ODS [4 mm × 30 cm (Micropak MCH-10)] were purchased from Varian Aerograph, Palo Alto, CA.

Materials. Absolute ethanol and 95% ethanol were purchased from U.S. Industrial Chemical Co., New York, NY. Methylene chloride, ethyl acetate, acetonitrile, petroleum ether, hexane, and diethyl ether were obtained from Fisher Scientific Co., Fairlawn, NJ. α-Tetralone, 2,4-xylol, and 2,5-xylol were purchased from Aldrich Chemical Co., Milwaukee, WI. Benzophenone, 1-chloronaphthalene, *m*- and *p* cresols, and 95% hydrazine hydrate were purchased from Eastman Kodak Co., Rochester, NY. *o*-Cresol, toluene (certified spectroanalyzed), and *p*-xylene (certified spectroanalyzed) were obtained from Fisher Scientific Co., Fairlawn, NJ. Ⓟ-Rose Bengal[61] was purchased from Hydron Laboratories, Inc., New Brunswick, NJ. *N*-Acetyl-L-tyrosine ethyl ester was purchased from ICN Pharmaceuticals, Inc., Cleveland, OH. *N*-Trimethylsilylimidazol in pyridine, available as "TRI-SIL-Z", was obtained from Pierce Chemical Co., Rockford, IL, and *N*-acetyl-L-phenylalanine ethyl ester was obtained from Sigma Chemical Co., St. Louis, MO.

General Photooxidation Procedure. The photolysis apparatus was patterned after one in the literature[62] and consisted of a Pyrex vessel equipped with a jacketed immersion well, a fritted gas inlet at the bottom, a gas outlet fitted with a Dewar condenser, and a side neck for the introduction of reactants. During photooxygenation the photolysis apparatus was immersed in an ice water bath and the reaction mixture was maintained at ca. 5 °C by circulating ice water through the jacket of the immersion well. Irradiation was carried out by means of a General Electric DWY 650 Watt lamp.

Preparation of Benzophenone Hydrazone. A mixture of 25.2 g (0.138 mol) of benzophenone, 10 g (0.2 mol) of 95% hydrazine hydrate, and 15 mL of absolute ethanol was refluxed for 10 h. Benzophenone hydrazone was recrystallized from 95% ethanol to give 22.0 g (80%) of long white needles, mp 97–98 °C (lit.[63] mp 98 °C).

Preparation of Active Manganese Dioxide.[64] A solution of 550 g of manganese sulfate tetrahydrate in 750 mL of water and a 40% aqeous solution of sodium hydroxide (1170 mL) were added simultaneously during 1 h to a hot, stirred solution of 480 g of potassium permanganate in 3 L of water. Stirring was continued for an additional hour. The precipitated manganese dioxide was then filtered off and washed with water until the washings were colorless. The solid was dried in an oven at 100–120 °C and ground to a fine powder (450 g) before use.

Preparation of Diphenyldiazomethane.[65] A solution of 5.8 g (30 mmol of benzophenone hydrazone, 3.0 g of anhydrous magnesium sulfate, and 60 mL of methylene chloride was stirred in an ice water bath. To this rapidly stirred solution was added, in one portion, 9.2 g of active manganese dioxide. Stirring was continued for 2 h at 0 °C and then for 1 h at room temperature. Solid material was filtered off and washed with methylene chloride. Removal of the solvent by rotary evaporaton gave a dark, maroon-colored oil which, upon cooling, gave 5.2 g (88%) of red needles of diphenyldiazomethane, mp 30 °C (lit.[65] mp 35 °C).

Photosensitized Oxidation of Diphenyldiazomethane in the Presence of 1-Chloronaphthalene. A solution of 24 g (148 mmol) of 1-chloronaphthalene in 500 mL of acetonitrile (ACS reagent grade) was placed in the photolysis apparatus and to it was added 400 mg of Ⓟ-Rose Bengal[61] as sensitizer. Irradiation was begun and oxygen was bubbled through the solution via the fritted inlet. A solution of 0.95 g (4.9 mmol) of diphenyldiazomethane in 50 mL of acetonitrile was introduced in 10-mL aliquots at 5-min intervals during the photolysis. Irradiation was terminated after 30 min at which time the color of the diazo compound was no longer detectable.

Following removal of the solvent by rotary evaporation, the reaction mixture residue was dissolved in ether and shaken with 5% sodium hydroxide. The aqueous layer was separated, acidified with 5% hydrochloric acid, and then shaken with ether. The ether layer was separated and the ether evaporated on the rotary evaporator. The residue, containing mainly phenolic compounds, was then subjected to preparative TLC on 20-cm × 20-cm, 0.25-mm-thick, silica gel plates with benzene as developing solvent. Fractions with R_f values of 0.55, 0.45, and 0.33 were removed from the plates. These fractions, consisting mainly of various chloronaphthols, were further purified by GLC with use of a 20 ft × $^3/_4$ in. 15% SE-30 on Chromosorb W column (column temperature 180 °C. He flow rate 80 mL/min). GLC analysis of the TLC fraction with R_f 0.55 revealed it to be a mixture of two compounds with GLC retention times of 59 and 65 min. The component with a retention time of 59 min was identified as 2-chloro-1-naphthol by comparison of its GLC retention time and TLC R_f value with those of the authentic compound.

(58) Kotin, P.; Falk, W. L.; Mader, P.; Thomas, M. *Arch Ind. Hyg.* **1954**, *9*, 153.
(59) Hueper, W. C.; Kotin, P.; Tabor, E. C.; Payne, W. W.; Falk, H. L.; and Sawicki, Z. *Arch. Pathol.* **1962**, *74*, 89.
(60) Epstein, S. S.; Joshi, J.; Andrea, J.; Mantel, N.; Sawicki, E.; Stanley, T.; Tabor, E. C. *Nature (London)* **1966**, *212*, 1305.
(61) Blossey, E. C.; Neckers, D. C.; Thayer, A. L.; Schaap, A. P. *J. Am. Chem. Soc.* **1973**, *95*, 5820.
(62) Foote, C. S.; Wexler, S.; Ando, W.; Higgins, R. *J. Am. Chem. Soc.* **1968**, *90*, 975.
(63) Miller, J. B. *J. Org. Chem.* **1959**, *24*, 560.
(64) Attenburrow, J.; Cameron, A. F. B.; Chapman, J. H.; Evans, R. M.; Hems, B. A.; Jansen, A. B. A.; Walker, T. *J. Chem. Soc.* **1952**, 1094.
(65) Reimlinger, H. *Ber.* **1954**, *97*, 3493.

The product had mp 62–63 °C (lit.[66] mp 64–65 °C; mixture mp 61–62 °C). The mass spectrum and infrared spectrum of the product 2-chloro-1-naphthol were identical with those of the authentic compound. The component with a GLC retention time of 65 min was identified as 8-chloro-1-naphthol from its melting point [mp 62 °C (lit.[67] mp 66–67 °C (recorded on microscope hot stage)] and mass spectrum (m/e 178). The m/e of this compound was depressed when it was mixed separately with each of the other chloronaphthols isolated from the reaction mixture.

The TLC fraction with R_f 0.45 was further purified by GLC. Its retention time was 61 min. This product was identified as 1-chloro-2-naphthol from its melting point mp 68–69 °C (microscope hot stage) (lit.[68] mp 70 °C)] and its mass spectrum (m/e 178). The melting point of this compound was depressed when it was mixed separately with the other chloronaphthols obtained from the reaction mixture. The TLC fraction with R_f 0.33 was also further purified by GLC and had a retention time of 114 min. This material was identified as 4-chloro-1-naphthol from its melting point [mp 119–120 °C (microscope hot stage) (lit.[69] mp 121 °C)] and its mass spectrum (m/e 178). The melting point of this compound was depressed when mixed separately with the other chloronaphthols obtained from the reaction mixture. The percentage yields of the chloronaphthol products were determined by GLC analysis and peak integration (cut and weigh method) with peak calibration based on the authentic materials. The yields were found to be 0.8% each for 2-chloro-1-naphthol, 1-chloro-2-naphthol, and 8-chloro-1-naphthol and 4.3% for 4-chloro-1-naphthol. The total yield of chloronaphthol products was 6.7%.

A number of control reactions were run as follows. When the reaction is carried out as described above but without addition of diphenyldiazomethane then no chloronaphthols are formed. Similarly, carrying out the reaction with 1-chloronaphthalene and diphenyldiazomethane but without irradiation led to no chloronaphthol production. When the chloronaphthalene photooxidation is carried out with benzophenone[70] (0.95 g; 5.2 mmol; in 50 mL of acetonitrile) instead of diphenyldiazomethane then no chloronaphthol products are formed.

Preparation of α,α-Dichlorotetralone.[66] To a stirred solution of 20 g (0.14 mmol) of tetralone in 400 mL of methylene chloride was added over a period of 1 h a solution of 25.3 g (2.3 equiv.) of chlorine dissolved in 300 mL of methylene chloride. The reaction mixture was stirred for an additional 2 h and then washed thoroughly with water, sodium bicarbonate, and water again. The solvent was evaporated and the yellow solid remaining was recrystallized from an either–petroleum ether mixture to give α,α-dichlorotetralone, mp 74–75 °C (lit.[66] mp 74–75 °C), in 85% yield (25 g).

Preparation of 2-Chloro-1-naphthol.[66] In a 500-mL three-necked round-bottom flask fitted with a mechanical stirrer and a reflux condenser carrying a calcium chloride drying tube was placed 7.0 g (0.3 mol) of freshly cut, small pieces of sodium. To this was added 70 mL of anhydrous methanol at such a rate as to maintain a vigorous reflux. When all of the sodium was dissolved stirring was discontinued and a solution of 17.8 g (0.1 mol) of α,α-dichlorotetralone in 200 mL of anhydrous methanol was added. The reaction mixture was refluxed for 3 h cooled, and then poured into 1 L of water. The mixture was then acidified with dilute hydrochloric acid. The solid which precipitated was filtered off and dried to give 13 g (95% yield) of crude 2-chloro-1-naphthol. The crude product was recrystallized from petroleum ether to give white crystals with mp 64–65 °C (lit.[66] mp 65–65 °C).

Photosensitized Oxidation of Diphenyldiazomethane in the Presence of Toluene. A solution of 50 mL (62 mmol) of toluene (ACS spectroanalyzed grade) in 500 mL of methylene chloride was placed in the photolysis apparatus and to it was added 400 mg of Ⓟ-Rose Bengal sensitizer. Oxygen bubbling and irradiation were begun and a solution of 0.95 g (4.9 mmol) of dephenyldiazomethane in 50 mL of methylene chloride was introduced in 10-mL aliquots at 5-min intervals during the photolysis. Irradiation was terminated after 30 min at which time the color of the diazo compound was no longer visible. Methylene chloride was removed by rotary evaporation and the residue was then dissolved in ether and the ether solution shaken with 5% sodium hydroxide. The aqueous layer was separated, acidified with 5% hydrochloric acid, and then shaken with ether. The ether layer was separated and the ether evaporated. The residue (containing mainly phenolic compounds) was subjected to preparative TLC on 20-cm × 20-cm, 0.25-mm-thick, silica gel plates with benzene as developing solvent. The product cresols were extracted from the TLC plates with ether and were further purified by GLC with use of a 3% tricresylphosphate on 100–200 mesh Gas Chrom Q column ($^1/_4$ in. × 15 ft, 135 °C, He flow rate = 60 mL/min). The GLC retention times of the *o*-, *m*-, and *p*-cresols were 37.5, 51, and 47.5 min, respectively. The cresols were identified by comparison of TLC R_f, IR, mass-spectral, and GLC retention time data with those of the appropriate authentic material. The percentage yields of the cresols were determined by GLC peak area with use of authentic compounds for calibration. The yields were found to be 1.3% for *o*-cresol, 0.8% for *m*-cresol, and 0.9% for *p*-cresol.

Control reactions indicated the following: (1) no cresols are formed in the absence of diphenyldiazomethane, (2) no cresols are formed in the absence of irradiation, and (3) no cresols are formed when 0.95 g (5.2 mmol) of benzophenone was substituted for diphenyldiazomethane.

Photosensitized Oxidation of Diphenyldiazomethane in the Presence of *p*-Xylene. A solution of 6 mL (50 mmol) of *p*-xylene in 350 mL of acetonitrile (reagent grade) was placed in the photolysis apparatus and to it was added 100 mL of Ⓟ-Rose Bengal. Oxygen was bubbled through the fritted inlet and irradiation begun. A solution of 0.98 g (5 mmol) of dephenyldiazomethane in 50 mL of acetonitrile was then introduced into the reaction vessel. Irradiation was continued for 30 min at which time the color of the diazo compound was absent. The solvent was removed by rotary evaporation and the residue dissolved in diethyl ether and then extracted with 5% aqueous sodium hydroxide. The aqueous layer was separated, acidified with 5% HCl, and then extracted with ether. The organic layer was separated and the ether removed by rotary evaporation. The residue was subjected to TLC on 20- × 20-cm, 0.25-mm-thick, silica gel plates with use of benzene as developing solvent. The TLC fraction with R_f 0.3 was shown to contain 2,5-xylol and 2,4-xylol (NIH shift product). The xylols were further purified by GLC with use of a 6% tricresylphosphate on 100–200 mesh Gas-Chrom Q column ($^3/_8$ in. × 20 ft, column temperature 120 °C the flow rate = 100 mL/min. The GLC retention times were 240 and 265 min for 2,5-xylol and 2,4-xylol, respectively. The xylols were identified by comparing their GLC retention times and IR spectra with those of the authentic compounds. The trimethylsilyl ethers of the xylols were prepared by stirring material from the TLC extract with *N*-trimethylsilylimidazole. The trimethylsilyl ethers were separated by GLC on a 4% OV-17 on 60–80 mesh Chromsorb W column ($^1/_4$ in. × 6 ft, column temperature 80 °C, He flow rate = 60 mL/min). The GLC retention times of the trimethylsilyl ethers of 2,5-xylol and 2,4-xylol were 25 and 28 min, respectively. The GLC retention times and mass spectral data of the trimethylsilyl ethers were identical with those of the authentic compounds. The yields of 2,5-xylol and 2,4-xylol were 2.1 and 0.4%, respectively.

Control experiments indicated the following: (1) no xylols were formed in the absence of diphenyldiazomethane or in the absence of irradiation and (2) no xylols are formed when 0.95 g (5.2 mmol) of benzophenone was used instead of diphenyldiazomethane.

Photosensitized Oxidation of Diphenyldiazomethane in the Presence of *N*-Acetyl-L-phenylalanine Ethyl Ester. A solution of 1.4 g (7 mmol) of *N*-acetyl-L-phenylalamine ethyl ester in 75 mL of acetonitrile (ACS reagent grade) was placed in the photolysis apparatus and to it was added 200 mg of Ⓟ-Rose Bengal as sensitizer. Oxygen was bubbled through the solution via the fritted inlet and irradiation was begun. A solution of 0.98 g (5 mmol) of dephenyldiazomethane in 50 mL of acetonitrile was introduced in 10-mL aliquots at 5-min intervals during the photolysis. Irradiation was discontinued after 30 min at which time the color of the diazo compound was no longer present. The reaction mixture was analyzed by two methods. In the first method the reaction mixture was concentrated to ca. 15 mL, 5 g of silica gel (80–100 mesh) was added as adsorbent, and the residual solvent was removed in vacuo. The silica gel coated with the reaction mixture was transferred to a chromatography column containing 400 g of silica gel (80–100 mesh) and was chromatographed with use of successive mixtures of ethyl acetate (0–50%) in methylene chloride as eluants. Fractions were collected with use of an automatic fraction collector and were subjected to analytical TLC with use of a mixture of ethyl acetate and methylene chloride (1:2) as developing solvent. Fractions having the same TLC R_f value as authentic *N*-acetyl-L-tyrosine ethyl ester were collected and further purified by preparative TLC on 20- × 20-cm, 0.25-mm thick, silica gel plates with use of a mixture of ethyl acetate and methylene chloride (1:2) as eluting solvent. The tyrosine derivative was removed from the TLC plates with use of methanol.

In the second workup method acetonitrile was removed by rotary evaporation and the residue subjected to TLC analysis on 20- × 20-cm, 0.25-mm thick, silica gel plates with use of a mixture of ethyl acetate and methylene chloride (1:1) as developing solvent. The TLC fraction with the same R_f value as authentic *N*-acetyl-L-tyrosine ethyl ester was re-

(66) Sterens, C. L.; Beereboom, J. J.; Rutherford, K. G. *J. Am. Chem. Soc.* **1955**, *77*, 4590.
(67) Heilbron, I.; Bunbury, H. M., Eds. "Dictionary of Organic Compounds"; Eyre and Spottiswoode: London, 1953; Vol. 1, p 529.
(68) Morgan, G. T.; Burstall, F. H. *J. Chem. Soc.* **1928**, 3269.
(69) Buu-Moi, Ng, Ph. *Recl. Trav. Chim Pays-Bas* **1954**, *73*, 197.
(70) This control reaction is necessary since it has been shown that these conditions lead to olefin epoxidation in some cases. Shimizu, N.; Bartlett, *J. Am. Chem. Soc.* **1976**, *98*, 4193.

moved from the plates with methanol. The tyrosine derivative was further purified by HPLC with use of a reverse phase ODS column (30 cm × 4 mm) and by eluting (2 mL/min) at room temperature with 35% methanol in water. *N*-Acetyl-L-tyrosine ethyl ester was identified by comparing its TLC R_f, HPLC retention time, IR, and mass-spectral data with those of the authentic material. The product had mp 77-78 °C (lit.[71] mp 79-80 °C. The yield was determined by injecting known amounts into the liquid chromatograph followed by integration (cut and weigh method of the resulting peaks. The yield of *N*-acetyl-L-tyrosine ethyl ester was 1%.

In separate experiments it was shown that no tyrosine derivative was produced in the absence of diphenyldiazomethane, or when the procedure is carried out without irradiation, or when 0.95 g (5.2 mmol) of benzophenone is substituted for diphenyldiazomethane.

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(71) Kaufman, S.; Neurath, H.; Schwert, G. W. *J. Biol. Chem.* **1949**, *177*, 793.

Intramolecular Excimer Formation with 1,3-Di(1-pyrenyl)propane. Decay Parameters and Influence of Viscosity

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Abstract: The fluorescence decays, studied as a function of temperature, of 1,3-di(1-pyrenyl)propane undergoing intramolecular excimer formation in toluene and other solvents can be fitted to a sum of three exponentials. The decay times have similar values for the monomer and the excimer at temperatures above 20 °C. Data showing that the decay parameters and the excimer-to-monomer fluorescence intensity ratio are strongly influenced by solvent viscosity are presented.

Introduction

Recently Thistlethwaite et al.[1] reported on the kinetics of intramolecular excimer formation with 1,3-di(1-pyrenyl)propane (Py(3)Py) and 1,10-di(1-pyrenyl)decane (Py(10)Py) in a number of solvents, based on time-correlated single-photon counting measurements at one temperature (20 °C). They concluded from their data that for Py(3)Py the excimer formation rate does not show a marked dependence on solvent bulk viscosity. This conclusion is in conflict with results obtained before using Py(3)Py and similar bichromophoric systems.[2-5] With these probe molecules, for example, the viscosity-dependent main-phase transition and the pretransition in phosphatidylcholine bilayers could readily be detected. In other studies the fluidity of micelles and biological membranes was investigated as a function of temperature. As the impression could have been made[1] that fluorescence studies with Py(3)Py do not give meaningful results on the fluidity of media such as biological membranes, we present here experimental data confirming that the kinetic parameters of Py(3)Py indeed strongly depend on solvent viscosity. Further, the kinetic model and the data published by Thistlethwaite[1] will be discussed in some detail and it will be shown that an accurate determination of the triple-exponential fluorescence decay parameters is difficult at 20 °C for Py(3)Py in solvents such as toluene, due to the small values of the amplitude ratios of the three exponentials.

Experimental Section

The 1,*n*-di(1-pyrenyl)alkanes Py(*n*)Py with $n = 3$ and 10 were synthesized as described before[6] and were purified by high-pressure liquid chromatography (HPLC). The alkane solvents (Merck) hexane, dodecane, hexadecane, and cyclohexane were purified by chromatography over SiO_2/Al_2O_3. Tetrahydrofuran (Merck, Uvasol) was refluxed over potassium in a nitrogen atmosphere. Toluene (Merck, for fluorescence spectroscopy) and the alcohols (Merck, Uvasol or the best commercially available quality) were used as received. The solutions, lower than 10^{-5} M, were degassed by the freeze-pump-thaw technique (5 cycles). The fluorescence decays were measured employing time-correlated single-photon counting.[7] As an excitation source a nitrogen-filled (1 atm) flash lamp (Edinburgh Instruments, 199 F) was used, operating at 100 kHz. The half-width of the pulse had a value between 3 and 4 ns. Under these conditions the lamp proved to be stable over periods up to 100 h. Single exponential decay times down to 500 ps can be accurately determined with this apparatus.[8] The scatter solution (Ludox, Du Pont) and the sample were alternatively positioned in the light path until 1000 counts

(1) Snare, M. J.; Thistlethwaite, P. J.; Ghiggino, K. P. *J. Am. Chem. Soc.* **1983**, *105*, 3328.
(2) Zachariasse, K. A. *Chem. Phys. Lett.* **1978**, *57*, 4299.
(3) Johnson, G. E. *J. Chem. Phys.* **1975**, *63*, 4047. Emert, J.; Behrens, C.; Goldenberg, M. *J. Am. Chem. Soc.* **1979**, *101*, 771. Turro, N. J.; Aikawa, M.; Yekta, A. *Ibid* **1979**, *101*, 772. Dangreau, H.; Joniau, M.; De Cuyper, M. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 468. Georgescauld, D.; Desmasez, J. P.; Lapouyade, R.; Babeau, A.; Richard, H.; Winnik, M. A. *Photochem. Photobiol.* **1980**, *31*, 539. Zachariasse, K. A.; Kühnle, W.; Weller, A. *Chem. Phys. Lett.* **1980**, *73*, 6. Melnick, R. L.; Haspel, H. C.; Goldenberg, M.; Greenbaum, L. M.; Weinstein, S. *Biophys. J.* **1981**, *34*, 499. Zachariasse, K. A.; Vaz, W. L. C.; Sotomayor, C.; Kühnle, W. *Biochim. Biophys. Acta* **1982**, *688*, 323. Dangreau, H.; Joniau, M.; De Cuyper, M.; Hanssens, I. *Biochemistry* **1982**, *21*, 3594. Almeida, L. M.; Vaz, W. L. C.; Zachariasse, K. A.; Madeira, V. M. C. *Ibid.* **1982**, *21*, 5972. Zachariasse, K. A.; Kozankiewicz, B.; Kühnle, W. *Laser Chem.*, in press. Zachariasse, K. A.; Kozankiewicz, B.; Kühnle, W. In "Surfactants in Solution"; Mittal, K. L., Ed.; Plenum Press: New York, 1983.
(4) Zachariasse, K. A. In "Fluorescent Techniques and Membrane Markers in Cancer and Immunology: Membrane Dynamics, Cellular Characterization and Cell Sorter"; Viallet, P., Ed.; Elsevier: Amsterdam, 1984.
(5) Zachariasse, K. A.; Kühnle, W.; Weller, A. *Chem. Phys. Lett.* **1978**, *59*, 375.
(6) Zachariasse, K. A.; Kühnle, W. *Z. Phys. Chem. (Wiesbaden)* **1976**, *101*, 267.
(7) Ware, W. R. In "Creation and Detection of the Excited State"; Lamola, A. A., Ed.; Marcel Dekker: New York, 1971; Vol IA, p 213.
(8) Busse, R.; Striker, G.; Szabo A,; Zachariasse, K. A., work in progress.

0002-7863/84/1506-1045$01.50/0