excess of hydrogen at 50 lb. per square inch for four hours. The crude reaction mixture was filtered through Celite to remove the majority of the catalyst. The filtrate was concentrated *in vacuo* and the sirupy residue taken up in 200 ml. of distilled water and freeze-dried.

The crude reaction mixture was filtered through Celite to remove the majority of the catalyst. The filtrate was concentrated *in vacuo* and the sirupy residue taken up in 200 ml. of distilled water and freeze-dried.

The freeze-dried material from five such runs was combined and dissolved in 450 ml. of butanol saturated with water. The *p*H was adjusted to 2.0 and the solution filtered. The filtrate was chromatographed on a 6-inch column packed with 3.4 kg. of acid-washed Celite using a 80% BuOH–20% $CHCl_3$–*p*H 2.0 developer. A total of twelve 250-ml. cuts were taken. The composition of the cuts was determined by paper chromatography using a butanol–phosphate–*p*H 2.0 system.

Cuts 1 and 2 were essentially pure 6-deoxy-6-demethylchlorotetracycline. Cuts 3 through 9 were a mixture of 6-deoxy-6-demethylchlorotetracycline, 6-demethyl-6-deoxytetracycline and a small amount of starting material. Cuts 3 through 9 were combined and concentrated to 400 ml. The *p*H was adjusted to 2.0 and the solution filtered. The clear filtrate was columned as before. Paper strips indicated that cuts 1 through 7 were essentially pure 6-deoxy-6-demethylchlorotetracycline.

Cuts 1 and 2 from the first column and cuts 1 through 7 from the second column were concentrated to dryness *in vacuo*. The residue was taken up in 300 ml. of water the *p*H adjusted to 2.0 with hydrochloric acid and filtered. The filtrate was freeze-dried to yield 4.85 g. of 6-deoxy-6-demethylchlorotetracycline hydrochloride.

Further purification was accomplished as follows: 2.3 g. of the freeze-dried material was dissolved in 55 ml. of methanol and the hazy solution filtered. The *p*H of the clear filtrate was adjusted to 9.0 by the addition of triethylamine and then brought down to *p*H 1.5 by the addition of concentrated sulfuric acid. Upon stirring and scratching, bright yellow needles deposited. The yield of 6-deoxy-6-demethylchlorotetracycline (**31**) was 1.8 g.

Anal. Calcd. for $C_{21}H_{21}O_7ClN_2.H_2SO_4.H_2O$: C, 44.6; H, 4.46; N, 4.96; Cl, 6.28. Found: C, 44.60; H, 4.45; N, 4.75; Cl, 6.51.

***Dedimethylamino-6-demethyl-6,12a-dideoxychlorotetracycline* (17).**—Zinc dust (500 mg.) was added to a solution of 6-deoxy-6-demethylchlorotetracycline (400 mg.) and anhydrous sodium acetate (65.5 mg.) in glacial acetic acid (20 ml.) and water (9 ml.). The mixture was stirred under nitrogen for 1.5 hours and an additional 500 mg. of zinc dust was added. After 6 hours the reaction mixture was diluted with 300 ml. of ethyl acetate, water (2:1) and the undissolved zinc filtered off. The aqueous layer was separated and extracted thrice with ethyl acetate. The combined ethyl acetate layers were washed with 1 *N* sulfuric acid and thrice with water. The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo*. The residual orange solid was slurried in 10 ml. of toluene and again concentrated to dryness *in vacuo*. The yield of crude dedimethylamino-6-demethyl-6,12a-dideoxychlorotetracycline (**17**) was 97 mg. An analytical sample was obtained by washing with a small volume of chloroform. The infrared spectrum, the ultraviolet spectrum ($\lambda_{max}^{0.00\ N\ \mathrm{NaOH(MeOH)}}$ 495, 469, 375, 262 mμ) and the chromatographic behavior (R_f 0.61 in a butyl acetate–formamide–acetone system using paper buffered at *p*H 6.0) of this material were identical to the corresponding properties of the synthetic amide **17**.

Anal. Calcd. for $C_{19}H_{16}NClO_6$: C, 58.6; H, 4.14; N, 3.60. Found: C, 58.69; H, 4.64; N, 3.88.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY, NEW YORK 58, N. Y.]

Ozonolysis of Polycyclic Aromatics. VIII.[1] Benzo[a]pyrene[2,3]

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Ozonization of benzo[a]pyrene (I) in methylene chloride and 3:1 methylene chloride–methanol with one molar equivalent of ozone produced a mixture of quinones from which could be isolated benzo[a]pyrene-3,6-dione (II) and benzo[a]pyrene-1,6-dione (III) in 1:3 ratio, and trace amounts of benzo[a]-pyrene-4,5-dione. No solvent effects were observed. Ozonolysis of I with two molar equivalents of ozone gave 7H-benz[de]anthracen-7-one-3,4-dicarboxylic acid (IV) while four, six and eight molar equivalents of ozone produced only 1,2-anthraquinonedicarboxylic acid (VII). In all cases unreacted I was recovered. Ozonolysis of the quinone mixture also produced IV and VII. The stoichiometry of these various ozonizations is unclear but does suggest predominant attack at positions 1, 3 and 6 in I by more than one molecule of ozone. Several mechanisms are proposed. There now does not seem to be any simple correlation between K- and L- region additivity toward ozone and carcinogenicity in the series anthracene, benz[a]anthracene, dibenz[a,h]anthracene and I.

Introduction

Since our original investigation of the ozonolysis of phenanthrene,[4a,4b] we have actively investigated the reaction between ozone and polycyclic aromatic hydrocarbons. Our purpose was twofold: (i) to elucidate the nature of ozone attack on aromatic systems; and (ii) to correlate the relative carcinogenicity of these polycyclics with the course and mechanism of the ozonolysis reaction. Published results of our studies on naphthacene,[4c] benz[a]anthracene[4d,4e] and dibenz[a,h]anthracene[1,4f] have led us to note a simple correlation between carcinogenicity and K- and L-region activity toward ozone in this limited series.[4c,4f] In this paper we report on the ozonization of the strong carcinogen (++++)[5] benzo[a]pyrene (I).[6]

Results

Ozonization of I in methylene chloride, and methylene chloride–methanol (3:1) with one molar equivalent of ozone (3.5 vol. %) at −70°, and at 20°, gave a mixture of benzo[a]pyrene-3,6-dione (II), benzo[a]pyrene-1,6-dione (III) and benzo[a]-

(1) Part VII, E. J. Moriconi, W. F. O'Connor, W. J. Schmitt, G. W. Cogswell and B. P. Fürer, *J. Am. Chem. Soc.*, **82**, 3441 (1960).

(2) Presented in part at the Symposium on Ozone Chemistry, 135th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1959.

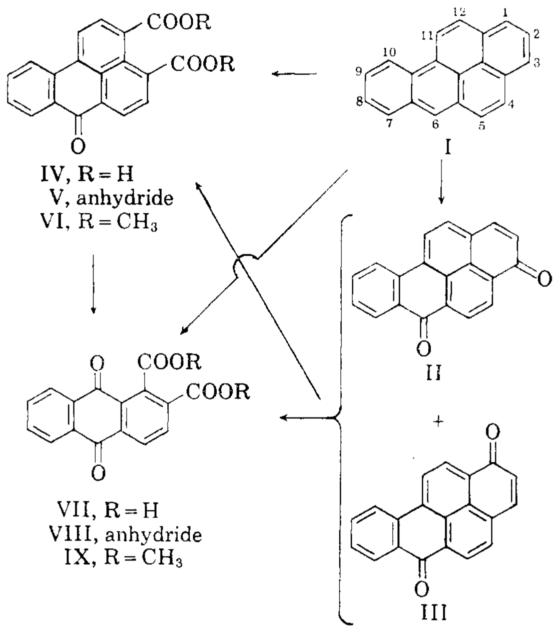
(3) This research was supported by a grant C-3325(C3) from the U. S. Public Health Service, National Cancer Institute.

(4) (a) W. J. Schmitt, E. J. Moriconi and W. F. O'Connor, *J. Am. Chem. Soc.*, **77**, 5640 (1955); (b) W. F. O'Connor, W. J. Schmitt and E. J. Moriconi, *Ind. Eng. Chem.*, **49**, 1701 (1957); (c) E. J. Moriconi, W. F. O'Connor and L. B. Taranko, *Arch. Biochem. and Biophys.*, **83**, 283 (1959); (d) E. J. Moriconi, W. F. O'Connor and F. T. Wallenberger, *Chemistry and Industry*, 22 (1959); (e) E. J. Moriconi, W. F. O'Connor and F. T. Wallenberger, *J. Am. Chem. Soc.*, **81**, 6466 (1959); (f) E. J. Moriconi, G. W. Cogswell, W. J. Schmitt and W. F. O'Connor, *Chemistry & Industry*, 1591 (1958).

(5) G. M. Badger, *Brit. J. Cancer*, **2**, 309 (1948).

(6) L. F. Fieser and F. C. Novello [*J. Am. Chem. Soc.*, **62**, 1855 (1940)] have previously ozonized I "but the material appeared either to remain unchanged or to be attacked extensively, and no satisfactory products were isolated."

pyrene-4,5-dione in 25–30% yield. Unreacted I was recovered in 60–65% yield. The quinone mixture, m.p. 245–246°, contained 22% of II, 67% of III and trace amounts (<1%) of the 4,5-dione. This mixture was identical with that obtained by Vollmann and co-workers,[7] and Cook and Hewett[8] by, respectively, sodium dichromate–acetic acid and potassium dichromate–sulfuric acid oxidation of I. Most probably, this mixture was also obtained on chromium trioxide–acetic acid oxidation of benzo[a]pyrene-1-carboxylic acid.[9] Cook and co-workers[10a] have chromatographically separated the mixture into II (red needles, m.p. 283–284° dec.) and III (yellow needles, m.p. 286° dec.) and decisively established the structure of each by independent synthesis from, respectively, 3-hydroxybenzo[a]pyrene and 1-methoxybenzo[a]pyrene. We have separated the mixture into II, III and benzo[a]pyrene-4,5-dione by reductive acetylation, fractional separation of the 3,6-, 1,6- and 4,5-diacetates, followed by hydrolysis and oxidation. Similar ozonization experiments with molar equivalent ozone absorption but lower ozone concentration (0.2 vol. %), or ozone–nitrogen streams, gave identical yields of the 245–246° mixture. Oxygen alone had no effect on I. No solvent effects were observed in methylene chloride and 3:1 methylene chloride–methanol. Ozonolysis of I at −70° with two and four molar equivalents of ozone, followed by alkaline hydrogen peroxide oxidation gave, respectively, 7H-benz[de]anthracene-7-one-3,4-dicarboxylic acid (IV) in 27% yield, and 1,2-anthraquinonedicarboxylic acid (VII) in 17% yield; 60% of unreacted I was recovered in both instances. Ozonolysis of I, however, with six and eight molar equivalents (−70°) gave only VII, in 22% and 29% yield, respectively, with decreasing amounts of unreacted I (Table I). Ozonolysis of the 245–246° mixture with one and three molar equivalents of ozone followed by alkaline hydrogen peroxide oxidation, gave, respectively, IV in 64% and VII in 43% yields. Similarly, IV was converted (with two molar ozone equivalents) to VII in 57% yield. Compounds I, II, III, the 4,5-dione, IV and VII are all inert to alkaline hydrogen peroxide.



IV, R = H
V, anhydride
VI, R = CH_3

I

II

+

III

VII, R = H
VIII, anhydride
IX, R = CH_3

Experimental[12]

The ozonator used in this research was a Welsbach Corporation T-23 laboratory ozonator. Our ozonization procedure is described in an earlier publication.[4e]

Reagents.—Yellow benzo[a]pyrene (I), m.p. 176.5–177.5°, was supplied by L. Light and Co., Ltd., Poyle-Colnbrook, Bucks, England, and Nutritional Biochemicals Corp. The alumina was Woelm non-alkaline aluminum oxide, activity grade I. Fisher Scientific Co. certified grade methylene chloride and methanol were used as solvents without further treatment.

Concentration of Reagents.—For all experiments, 5.0 or 10.0 mmoles of I (1.26 g. or 2.52 g.), 245–246° mixture (1.41 g. or 2.82 g.) and IV (1.50 g. or 3.00 g.) were dissolved in 350–500 ml. of solvent methylene chloride or 3:1 methylene chloride–methanol.

Ozonization of Benzo[a]pyrene (I) to the 245–246° Mixture.—A solution of I in methylene chloride was treated with ozone (0.2 vol. %, 3.5 vol. % and ozone–nitrogen[13]) at −70°,[14] or at 20°, until one molar equivalent (±0.1) had been absorbed. The resulting red solution was evaporated to dryness at room temperature. The crude dark amorphous material was dissolved in a minimum amount of benzene, adsorbed on a 35 × 2.5 cm. alumina-packed column and eluted with benzene. The yellow fluorescent (green in solution, blue on column under ultraviolet light) band of unreacted I was eluted first, followed successively by red and dark brown zones. Unreacted I (60–65%) was recovered by evaporation of the first 5 × 100 ml. of benzene eluate. The second, red zone was eluted with 6 × 100 ml. of 1:1 benzene-ethyl acetate, the combined fractions of which were evaporated to dryness. Recrystallization from methanol (charcoal) gave the mixture of diones as red microcrystals (25–30% yield), m.p. 245–246°. This product mixture was identical (combustion analyses, mixed m.p. and superimposable infrared spectra) with that, m.p. 245°, obtained by sodium dichromate–acetic acid oxidation of I.[7] We were unsuccessful in many attempts to separate chromatographically our 245–246° mixture and it was therefore used as such in its further ozonolyses to 7H-benz[de]anthracen-7-one-3,4-dicarboxylic acid (IV) and 1,2-anthraquinonedicarboxylic acid (VII).

By a reductive acetylation technique, Vollmann, *et al.*,[7] were able to separate the 245–246° mixture into III, m.p. 295°, and what was presumed to be II, m.p. 245°. Using the same technique, we have determined this mixture to contain II, III and trace amounts of benzo[a]pyrene-4,5-dione (<1%). The procedure follows:

A mixture of 3.78 g. (7.5 m*M* based on a mol. wt. of 282) of the 245–246° material, 7.5 g. of zinc dust in 100 ml. of acetic anhydride and a few drops of concentrated sulfuric acid was boiled for 30 min. The suspension was filtered to remove excess zinc and the orange solution was allowed to stand at *ca.* −5° for 2 days. The insoluble yellow material was filtered and recrystallized from xylene to give

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[7] H. Vollmann, H. Becker, M. Corell and H. Streeck, *Ann.*, **531**, 51, 130 (1937).

[8] J. W. Cook and C. L. Hewett, *J. Chem. Soc.*, 398 (1933).

[9] A. Windaus and K. Raichle, *Ann.*, **537**, 157 (1939), and Vollmann and co-workers[7] erroneously considered the red product, m.p. 244° and 245°, respectively, as the 3,6-dione. The pure quinone has a m.p. of 284°.[10,11]

[10] (a) J. W. Cook, R. S. Ludwiczak and R. Schoental, *J. Chem. Soc.*, 1112 (1950); (b) J. W. Cook and R. Schoental, *ibid.*, 170 (1948); (c) J. W. Cook and R. Schoental, *ibid.*, 47 (1950).

[11] Our work, see Experimental.

[12] Melting points below 360° were determined on a Kofler micro melting point apparatus; above 360° capillary m.p.'s are reported; all are corrected. Microanalyses were performed by the Schwarzkopf Micro-Analytical Laboratory, Woodside, N. Y. The infrared spectra (KBr pellet) were determined in a Perkin-Elmer model 21 spectrophotometer.

[13] G. A. Cook, A. D. Kiffer, C. V. Klumpp, A. H. Malik and L. A. Spence, Ozone Chemistry and Technology, "Advances in Chemistry Series" No. 21, Amer. Chem. Soc., Washington, D. C., 1959, pp. 44–52.

[14] Identical results were obtained using 3.5 vol. % and I dissolved in 3:1 methylene chloride–methanol at −70°.

3.48 g. of benzo[a]pyrene-1,6-diacetate, yellow needles, m.p. 243–244° (lit.[7] m.p. 245–246°). Its infrared spectrum showed a single strong C=O band at 5.66 μ.

Water was added to the acidic filtrate to precipitate a yellow amorphous solid which was filtered. Successive recrystallizations of this material from 5:1 ethanol–toluene, and then benzene gave 0.85 g. of benzo[a]pyrene-3,6-diacetate, m.p. 215–216° (lit.[10a] m.p. 215–216°).

The mother liquor from this second filtration was evaporated to dryness, and the resulting amorphous solid was triturated with acetone to give 0.75 g. of a yellow crude solid, m.p. 191–195°. One recrystallization from benzene raised the m.p. to 205–206°. Vollmann, *et al.*,[7] considered this material to be the benzo[a]pyrene-3,6-diacetate, and reported a m.p. of 204° but no analytical data. We believe these light yellow crystals to be a mixture of benzo[a]pyrene-3,6- and -4,5-diacetates on the basis of the following: This yellow solid was dissolved in a minimum amount of benzene, deposited on a 20 × 2.5 cm. alumina-packed column and eluted with benzene. The first 100-ml. fraction was evaporated to dryness, and the residue was recrystallized from benzene to give approximately 20 mg. of benzo[a]pyrene-*trans*-4,5-diacetate, m.p. 269–270° (the *cis*-diacetate has a m.p. of 214–215°[10b]). This compound showed an identical absorption spectrum and gave no depression on admixture with the benzo[a]pyrene-*trans*-4,5-diacetate mixture, m.p. 269–270° (from benzene), prepared from reductive acetylation of benzo[a]pyrene-4,5-dione; infrared spectrum: 5.67(s) μ (C=O).

Anal. Calcd. for $C_{24}H_{18}O_4$: C, 77.82; H, 4.89. Found: C, 77.80; H, 4.42.

Further elution of the column with 200 ml. of benzene gave 200 mg. of a yellow solid, m.p. 205–206° (from benzene), presumably a mixture of the 3,6- and 4,5-diacetates. Final elution of the column with 500 ml. of 1:1 benzene–ethyl acetate gave an additional 370 mg. of benzo[a]pyrene-3,6-diacetate, m.p. 215–216°, as yellow needles.

Each of the diacetates was hydrolyzed and the subsequent diol oxidized as follows: The diacetate was boiled with aq. alkali to which was added a small amount of sodium hydrosulfite. The solution was then filtered and oxidized with warm sodium dichromate–glacial acetic acid, after which it was permitted to stand for several hours at room temperature. On addition of water the insoluble quinone was separated by filtration.

Benzo[a]pyrene-3,6-dione (II), m.p. 288–289° (lit.[10a] m.p. 291° dec.), was obtained ultimately as red needles after successive recrystallizations from acetic acid (charcoal) and toluene in 0.85 g. (22%) yield; II gave a green solution in concd. sulfuric acid. Addition of water precipitated unchanged II. The infrared spectrum showed moderate C=O bands at 5.62 μ and 5.74 μ, a shoulder at 5.99 μ, and a strong band at 6.04 μ.

Anal. Calcd. for $C_{20}H_{10}O_2$: C, 85.09; H, 3.57. Found: 85.00; H, 3.50.

Benzo[a]pyrene-1,6-dione (III), m.p. 290–291° (lit.[10a] m.p. 292–293°), was obtained as orange needles from chlorobenzene (charcoal) in 2.52 g. (67%) yield; III gave a carmine-red solution in concd. sulfuric acid. Upon addition of water, the solution turned yellow-orange and precipitated unchanged III. The infrared spectrum showed a single strong C=O band at 6.04 μ. For comparative purposes, benzanthrone (Aldrich Chem. Co.,) resublimed to m.p. 171° (lit.[15] m.p. 167–168°) showed a strong C=O band at 6.00 μ.

Anal. Calcd. for $C_{20}H_{10}O_2$: C, 85.09; H, 3.57. Found: C, 85.10; H, 3.66.

Benzo[a]pyrene-4,5-dione, m.p. 253–254° (lit.[10a] m.p. 240°), was obtained as red needles from acetic acid; it showed no depression on admixture with an authentic sample and the infrared spectra were identical, showing a single strong C=O band at 5.96 μ.

The 4,5-dione gave a deep-violet solution with concd. sulfuric acid and reacted with *o*-phenylenediamine in acetic acid to give the quinoxaline derivative, m.p. 316–317° (lit.[10c] m.p. 318°); mixed m.p.'s 1:3 mixture of benzo[a]pyrene-3,6-(II) and 1,6-diones (III), 249–251°; 1:3 mixture of II and III, and traces of benzo[a]pyrene-4,5-dione: 245–246°, 247–248°.

The 205–206° diacetate mixture upon hydrolysis and oxidation in the usual manner gave a red solid which after recrystallization from benzene (charcoal) melted at 225–227°. We presume this to be a mixture of II and benzo[a]pyrene-4,5-dione since a 1:1 mixture of authentic II and the 4,5-dione had a m.p. of 225–227°.

Ozonolysis of I to 7H-Benz[de]anthracen-7-one-3,4-dicarboxylic Acid (IV).—The pale yellow solution of I in methylene chloride was treated with 2.0 ± 0.1 molar equivalents of ozone at −70°. Color changes occurring during ozone absorption were red after approximately one molar addition and yellow on completion. The solution was sparged with nitrogen to free it of excess oxygen and dissolved ozone. The addition of several ml. of methanol and a few drops of concentrated hydrochloric acid produced no precipitate nor did any appear on standing for several days at −10°, and then at ambient temperature. The solution was then evaporated to one-half its original volume at which time a pale yellow solid precipitated slowly which darkened on continued solvent removal. To the warm suspension was added additional methanol and the evaporation was continued until all the methylene chloride had been distilled; the final volume of the methanolic solution was 20–30 ml. This warm brown solution was treated with a mixture of 20% sodium hydroxide and 20 ml. of 30% hydrogen peroxide. The now green solution was acidified with concentrated hydrochloric acid to yield a yellow solution which deposited, on standing for several hours, a dark yellow solid. This crude material dissolved in the minimum amount of benzene was adsorbed on a 35 × 2.5 cm. alumina-packed column and eluted successively with benzene, ethyl acetate and methanol. The first 5 × 100-ml. benzene fractions were combined, and evaporated to dryness to yield a 60% recovery of I, m.p. 176–177°. The second, non-fluorescent, yellow zone was eluted successively with 200 ml. of 1:1 benzene–ethyl acetate, 200 ml. of ethyl acetate and finally with 200 ml. of 1:1 ethyl acetate–methanol; the combined fractions were evaporated to dryness to yield crude 7H-benz[de]anthracen-7-one-3,4-dicarboxylic acid (IV). This solid was dissolved in 10% sodium bicarbonate solution, charcoaled, filtered, and acidified to yield a bright yellow acid. A final crystallization from warm 1:1 acetone–water solution gave IV as bright yellow microcrystals in 27% yield; IV starts melting *ca.* 310° with gradual formation of the anhydride V, m.p. 366–367°. The m.p. range of IV is difficult to determine and consequently no m.p. has been previously reported. We made many attempts to isolate IV of analytical purity. The best results are reported below; infrared spectrum: (C=O's): 5.62m, 5.77s,[16a] 5.81–5.87s,[16c] 6.00s. Compound IV was identical (m.p. and mixed m.p. behavior, and superimposable spectra) with the acid obtained by the sodium dichromate–sulfuric acid oxidation (our work) of I, and the 245–246° mixture.

Anal. Calcd. for $C_{19}H_{10}O_5$: C, 71.70; H, 3.17. Found: C, 72.22; H, 3.25.

The ease of formation of the anhydride V prompted us ultimately to isolate the ozonolysis product of I, and the 245–246° mixture as V; V was quantitatively obtained from IV by dissolution in warm acetic anhydride, evaporation of excess solvent to one-half its original volume and filtration of the precipitated anhydride. One crystallization from nitrobenzene gave V as golden yellow plates, m.p. 366–367° (lit.[7] m.p. 364°); infrared spectrum: (C=O's) 5.52w, 5.59s, 5.74s and 5.99m; 6.23m.[16b]

Anal. Calcd. for $C_{19}H_{18}O_4$: C, 76.00; H, 2.68; Found: C, 76.04; H, 2.95.

Ozonolysis of the 245–246° Mixture to V.—The 245–246° dione mixture dissolved in methylene chloride was treated with 1.0 ± 0.1 molar equivalent of ozone at −70°, followed by the usual alkaline hydrogen peroxide–sodium hydroxide oxidation. Color changes of the solution observed during ozonization and general work-up procedure were the same as those for the ozonolysis of I to IV; V was obtained in 64% yield as bright yellow microcrystals m.p. 366–367° from nitrobenzene. A mixed m.p. of V, obtained from the ozonolysis of I and the 245–246° mixture, and the sodium dichromate–sulfuric acid oxidation of I,[7] showed no depression, and the infrared spectra were virtually superimposable.

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(15) (a) R. Scholl and C. Seer, *Ann.*, **394**, 143 (1912); (b) I. G. Farbenind.; Swiss Patent 134,622; German Patent 494,111; *Forschr. Theerfabrik. u. Industriezweige*, **16**, 1442 (1931).

(16) (a) Shoulder; (b) ring conjugated carbonyl; (c) broad band.

TABLE I

EFFECT OF OZONE MOLE RATIOS ON PRODUCTS AND YIELDS IN THE OZONIZATION OF I, THE 245–246° MIXTURE, AND IV IN METHYLENE CHLORIDE AT −70° WITH 3.5 VOL. % OZONE

Reactant	Mole ratio ozone: reactant	Unreacted I recovered, %	Yield, % 245–246° mixture	IV[b]	VII[b]
I[a]	1:1	60–65	25–30	..	..
I[a]	2:1	60	...	27	..
I[a]	4:1	60	...	..	17
I	6:1	33	...	..	22
I	8:1	18	...	..	29
245–246° mixt.[a]	1:1	..	...	64[c]	..
245–246° mixt.	3:1	..	...	..	43
IV	2:1	..	...	..	57

[a] See Experimental. [b] After alkaline hydrogen peroxide oxidation of initial ozonolysis products. [c] Isolated as the anhydride.[a]

N-Phenyl-7H-benz[de]anthracen-7-one-3,4-dicarboximide was obtained from V and aniline as yellow needles, m.p. 360–361° (lit.[15b] m.p. 360°) from acetic acid; infrared spectrum: (C=O's) 5.81s, 5.96s; 6.26m.[16b]

3,4-Dicarbomethoxy-7H-benz[de]anthracen-7-one (VI), m.p. 165–166°, bright yellow crystals from toluene, was obtained by mixing solutions of IV in dimethylformamide and diazomethane in ether at 0° and then permitting the solution to stand overnight at room temperature; infrared spectrum: (C=O's) 5.75s, 5.81s,[16a] 5.99s, 6.26m.[16b]

Anal. Calcd. for $C_{21}H_{14}O_5$: C, 72.83; H, 4.07; Found: C, 72.87; H, 3.91.

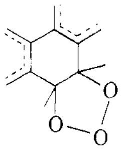
Ozonolysis of I to 1,2-Anthraquinonedicarboxylic Acid (VII).—A solution of I in methylene chloride was treated with 4.0 ± 0.1 molar equivalents of ozone at −70°. No precipitate appeared on standing at ambient temperature for several days and/or on addition of methanol and a few drops of concentrated hydrochloric acid. A pale yellow solid precipitated only on evaporation of the solution to about one-half its original volume; this solid darkened on further solvent removal. Upon evaporation to dryness the yellow-brown residue was triturated with 1:1 benzene–petroleum ether solution to yield a yellow solid, m.p. 130–140° dec. Over a period of several days this material darkened on standing in air, even at 0°. It burned brightly in an open flame. This unstable product was suspended in methanol and oxidized in customary fashion with hydrogen peroxide–sodium hydroxide solution. Acidification of the alkaline solution with concentrated hydrochloric acid gave 2.1 g. of a brown solid. This crude material, dissolved in the minimum amount of benzene, was adsorbed on a 35 × 2.5 cm., alumina-packed column, and eluted successively with benzene, 1:1 benzene–ethyl acetate, ethyl acetate, and 1:1 ethyl acetate–methanol. The first 6 × 100-ml. benzene fractions were evaporated to dryness to yield I, m.p. 176–177°, in 60% recovery. The second, pale yellow zone containing VII was eluted with benzene–ethyl acetate–methanol as previously indicated. As usual the chromatographic separation could be followed with ultraviolet light since VII showed no fluorescence. The combined fractions were evaporated to dryness to leave an amorphous residue which was dissolved in 10% sodium bicarbonate solution, charcoaled, filtered, and the filtrate acidified with concentrated hydrochloric acid to yield yellow crude VII, m.p. *ca.* 220°. Recrystallization from 1:1 acetone–water containing a few drops of hydrochloric acid gave 1,2-anthraquinonedicarboxylic acid (VII) in 17% yield, as pale yellow needles. On heating to *ca.* 230°, VII changed to an amorphous solid with gradual formation of the anhydride VIII, m.p. 318–319°. As previously noted,[4e] the m.p. range of VII is difficult to determine; lit.[17] m.p. of VII, 267–269°, and its anhydride VIII, 319–320°.[18] Compound VIII was prepared as previously reported from VII, m.p. 329–330°, (lit.[4e] m.p. 331–331.5°), and showed no depression on admixture with the 1,2-anthraquinonedicarboxylic acid anhydride obtained from the ozonolysis of benz[a]anthracene.[4e]

1,2-Dicarbomethoxyanthraquinone (IX), m.p. 209–211°, lit.[4e] m.p. 209–210.5°, obtained from VII and diazomethane in ethereal solution showed no depression on admixture with an authentic sample.

The effect on various ozone mole ratios on products and yields in the ozonization of I, the 245–246° mixture and IV are summarized in Table I.

Discussion

Mechanism.—It has been assumed that ozone can attack unsubstituted carbocyclic aromatics predominantly in two ways: (i) as a double bond reagent,[19] assuming a one-step[20] or nearly simultaneous electrophilic-nucleophilic[21], four-center attack on the most olefinic bond [*i.e.*, the bond with the lowest bond[20] (*ortho*)[22a] localization energy] to give initially cyclic five- (X) or four-membered (XI) (molozonides) primary ozonide[23,24] intermediates (or transition states) and ultimately bond cleavage products.[25] (ii) As an electrophilic reagent[34] assuming a two-step[35] attack at the most reactive centers [*i.e.*, the carbons with the lowest carbon localization energies[22a] (reactivity numbers)[22b] and/or the lowest paralocalization energies[20c,22a,36]] to give initially a σ-complex[37] (XII) followed by a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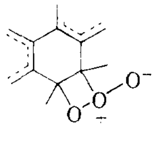
XI

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[17] R. Scholl and E. Schwinger, *Ber.*, **44**, 2992 (1911).

[18] R. Scholl and E. Neuberger, *Monatsh. Chem.*, **33**, 507 (1912).

[19] G. M. Badger, *J. Chem. Soc.*, 456 (1949); *Quart. Revs.*, **5**, 155 (1951); G. M. Badger and B. J. Christie in "Current Trends in Heterocyclic Chemistry," Butterworth Scientific Publications, 1958, pp. 1–7.

[20] (a) R. D. Brown, *J. Chem. Soc.*, 691, 3249 (1950); (b) R. D. Brown, *Quart. Revs.*, **6**, 63 (1952).

[21] R. Criegee, *Record Chem. Progr., Kresge–Hooker Sci. Lab.*, **18**, 111 (1957).

[22] (a) M. J. S. Dewar, *J. Am. Chem. Soc.*, **74**, 3357 (1952); (b) *Record Chem. Progr., Kresge–Hooker Sci. Lib.*, **19**, 1 (1958).

[23] See P. S. Bailey, *Chem. Revs.*, **58**, 925 (1958), for leading references.

[24] Most probably, crystalline, primary ozonides have been obtained by R. Criegee and G. Schroeder [*Ber.*, **93**, 689 (1960)] and P. de Bruyn [*Bull. soc. chim. Belg.*, **69**, 328 (1960)] on ozonization of, respectively, *trans*-di-*t*-butylethylene and 9,10-dimethylanthracene.

[25] As in indene,[26] naphthalene,[27] phenanthrene,[4a,4b,28,32b] acenaphthylene,[29] fluorene,[29,30] chrysene,[29,32b] triphenylene,[29,32b] pyrene,[31,32] benz[a]anthracene,[29,32b] benzo[b]fluorene[30] fluoranthrene,[29,33] perylene,[29] benzo[g]chrysene,[32] dibenz[c,g]phenanthrene,[32] picene[32] and dibenz[a,h]anthracene.[1,4f]

[26] J. L. Warnell and R. L. Shriner, *J. Am. Chem. Soc.*, **79**, 3165 (1957).

[27] P. S. Bailey and F. J. Garcia-Sharp, *J. Org. Chem.*, **22**, 1008 (1957).

[28] (a) P. S. Bailey and S. B. Mainthia, *ibid.*, **21**, 1089 (1956), and preceding papers; (b) J. P. Wibaut and Th. J. DeBoer, *Rec. trav. chim.*, **78**, 183 (1959).

[29] P. G. Copeland, R. E. Dean and D. McNeil, *Chemistry & Industry*, 329 (1959).

[30] P. G. Copeland, R. E. Dean and D. McNeil, *J. Chem. Soc.*, 3230 (1960).

[31] R. E. Dessy and M. S. Newman, *Org. Syntheses*, **38**, 32 (1958).

[32] (a) P. G. Copeland, R. E. Dean and D. McNeil, *Chemistry & Industry*, 98 (1960); (b) P. G. Copeland, R. E. Dean and D. McNeil, *J. Chem. Soc.*, 1232 (1961).

[33] R. H. Callighan, M. F. Tarker, Jr., and M. H. Wilt, *J. Org. Chem.*, **25**, 820 (1960).

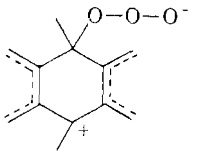
[34] J. P. Wibaut and F. J. L. Sixma, *Rec. trav. chim.*, **71**, 761 (1952), and preceding papers.

[35] (a) P. S. Bailey and J. B. Ashton, *J. Org. Chem.*, **22**, 98 (1957); (b) P. S. Bailey, *Chemistry & Industry*, 1148 (1957).

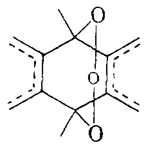
[36] E. C. Kooyman and J. A. Ketelaar, *Rec. trav. chim.*, **65**, 859 (1946).

[37] F. Dobinson and P. S. Bailey, *Tetrahedron Letters No. 13*, 14 (1960).

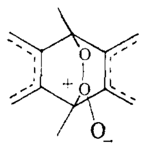
nucleophilic 1,4-addition to give primary ozonides XIII or XIV,[4e,38] and thence to *p*-quinones.[41] The insertion of a π-complex,[23,35b] or a charge-transfer complex[42,43] as precursors to primary ozonide formation is perhaps of theoretical interest but does not elucidate the nature and seeming disparity of ozone attack on aromatic systems. We have suggested that initial ozone attack involves, in the rate-controlling step, a transition state whose geometry corresponds to the most stable dihydro compound.[4e]



XII



XIII



XIV

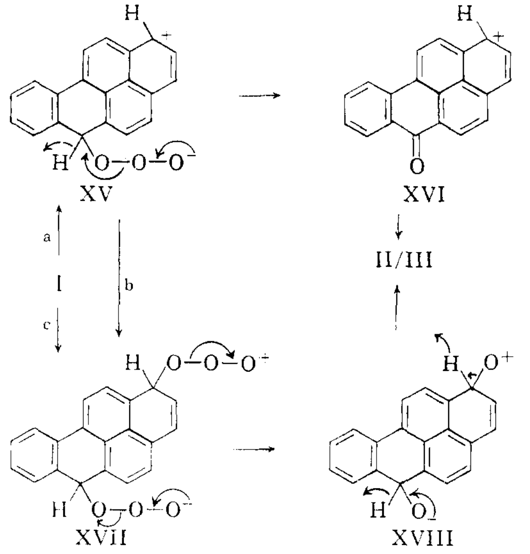
In I, M.O. calculations indicate the 6-, 1- and 3-positions (decreasing reactivity in this order) to have the lowest carbon localization energies,[22a] and the 4,5-bond to have the lowest bond localization energy[20a,44] (*o*-localization energy).[22a] Experimentally, there is ample evidence to support this localization theory of chemical reactivity.[45]

Any mechanism for the ozonization of I predominantly to II and III must account for the following facts: (a) Ozone attack occurs reluctantly, but almost exclusively, at the most reactive positions, 1, 3 and 6. (b) The 1,6- and 3,6-positions are spatially too far apart for the formation of cyclic primary ozonides such as XIII and XIV. (c) Although the stoichiometry of ozonization is difficult to follow since the initial ozonization products (II, III and IV) are more susceptible to ozonolysis than I, at least two molecules of ozone are required to form II and III. Both mechanistic pathways i and ii require 1:1 hydrocarbon–ozone stoichiometry.

In our view there are three principal ways in which ozone could attack I to form the initially observed diones, all of which are modifications of the type ii mechanism. (a) An initial electrophilic attack on carbon 6, 3, or 1 (probably 6) to give, for example, XV, which by loss of oxygen and hydride ion would yield the positively charged species XVI. Further nucleophilic attack by a second ozone molecule at the remaining reactive sites would yield ultimately II/III. (b) An initial electrophilic attack to give XV, followed by nucleophilic attack by a second ozone molecule to give XVII, which by loss of two molecules of oxygen, hydride ion and proton, would give II/III. (c) A concerted electrophilic–nucleophilic attack by two ozone molecules to give XVII, for example, followed by the same mechanistic sequence as in path b. Hydrogen ions could be removed as H_2O and H_2O_2 by ozone–oxygen.

Results obtained thus far, however, do not permit selection among a, b, or c as the more probable mechanism.

The fairly high measured oxidation–reduction potential of the 245–246° mixture is 0.437 v.[52] intermediate between such *p*-quinones as 1,4-naphthoquinone and 1,4-anthraquinone,[54] and would account for the resistance of I to the ozone oxidant. In support of nucleophilic attack by ozone, Bailey and co-workers[55] have recently demonstrated such attack by ozone on C-atoms of Schiff bases and nitrones, and we have additional evidence of nucleophilic attack in heterocyclics and their oxides.[56]



Although we found no solvent effect of the type of ozone attack on I, Dobinson and Bailey[37] have

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[38] P. S. Bailey and co-workers originally suggested this intramolecular cyclization when, as in anthracene[39] and 2,5-diphenylfuran,[40] the ends of the conjugated system are fixed and spatially close together so that ozone can reach across the ends of the system.

[39] P. S. Bailey and J. B. Ashton, *J. Org. Chem.*, **22**, 98 (1957).

[40] P. S. Bailey and H. O. Colomb, Jr., *J. Am. Chem. Soc.*, **79**, 4238 (1957).

[41] As in anthracene,[29,39] naphthacene[4c] and benz[a]anthracene.[4d,4e,32b]

[42] R. D. Brown, *J. Chem. Soc.*, 2224, 2232 (1959).

[43] A. Szent-Gyorgyi, I. Isenberg and S. L. Baird, Jr. [*Proc. Natl. Acad. Sci.*, **46**, 1444 (1960)] have made the interesting suggestion that carcinogenicity in polynuclear aromatic hydrocarbons may be connected in some way with their ability to form a strong charge-transfer complex with I_2.

[44] Brown[20a] has computed rates of bond reactions of various polycyclic hydrocarbons and predicted the 4,5-bond to be 120 times more reactive than the 11,12-bond; and in calculated idealized relative reaction rates, the predicted reactivity of the 4,5-bond is second only to the 6,7-bond in pentaphene.

[45] Compound I undergoes electrophilic substitution at the 6-position to form 6-nitro-,[46] 6-chloro-,[9] 6-formyl-,[47] 6-diazophenyl-,[48] 6-thiocyano-,[49] and probably 6-sulfonic acid.[46] Friedel–Crafts condensation with acetyl chloride gave the 1-isomer.[22a,47] Further, I undergoes rapid addition at the 4,5-bond with osmium tetroxide-pyridine,[50] but is inert to maleic anhydride.[8] Finally chromic acid oxidation leads to the 245–246° mixture.[7,9,10,51]

[46] A. Windaus and S. Renhak, *Z. physiol. Chem.*, **249**, 256 (1937).

[47] L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, **60**, 2542 (1938); **61**, 1565 (1939).

[48] L. F. Fieser and W. P. Campbell, *ibid.*, **60**, 1142 (1938).

[49] J. L. Wood and L. F. Fieser, *ibid.*, **63**, 2323 (1941).

[50] J. W. Cook and R. Schoental, *J. Chem. Soc.*, 170 (1948).

[51] D. S. Tarbell, E. G. Brooker, P. Seifert, A. Vanterpool, C. J. Claus and W. Conway, *Cancer Research*, **16**, 37 (1956).

[52] E. J. Moriconi, N. O. Smith, B. Rakoczy and W. F. O'Connor, unpublished information. Our value for benzo[a]pyrene-4,5-dione was 0.442 v. (lit.[53] 0.441 v.).

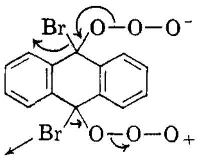
[53] G. M. Badger and H. A. McKenzie, *Nature*, **172**, 458 (1953).

[54] G. M. Badger, "Structures and Reactions of the Aromatic Compounds," Cambridge University Press, New York, N. Y., 1954, p. 89.

[55] A. H. Fiebel, R. E. Erickson, C. J. Abshire and P. S. Bailey *J. Am. Chem. Soc.*, **82**, 1801 (1960).

[56] E. J. Moriconi, F. Spano and W. F. O'Connor, unpublished information.

demonstrated a solvent effect in 9,10-dibromoanthracene, and possibly in anthracene itself. They have suggested initial electrophilic ozone attack to XII, followed by nucleophilic attack, intramolecularly to XIII. Alternatively, they too have conceived of nucleophilic attack by a second ozone molecule to give adducts such as XIX which would go to quinonoid products *via* the indicated electron distribution and loss of bromine, without oxidative workup. It is significant to note that in the four instances[37,41] in which type ii attack is presumed to occur, considerably less than 50% ozone could be accounted for on one molar equivalent ozone absorption. In the case of 9,10-dibromoanthracene, a 79–80% yield of anthraquinone was obtained on absorption of *two* molar equivalents of ozone. These facts are nicely accommodated by the formation of such intermediates as XVII and XIX *via* pathways a or b, or the oxidation of XVI by a second molecule of ozone.



XIX

Finally, the ozonolysis of II/III and IV must involve cleavage of partially localized double bonds which no longer fully participate in the aromatic system by virtue of quinone formation. These localized double bonds would be expected to be more susceptible to ozonolysis than the completely aromatic I.

Carcinogenicity and the Ozone Reaction.—Recently we noted a decrease in reactivity at the L-region toward ozone as an electrophilic reagent in the series anthracene > benz[a]anthracene > dibenz[a,h]-anthracene which was approximately paralleled by a corresponding increase in reactivity at the K-region toward ozone as a double bond reagent.[4d] This order which corresponded to a progressive increase in carcinogenic activity suggested that the strongly carcinogenic I, devoid of an L-region and with a very active K-region, should react strongly with ozone at the K-region. No such strong reaction was observed. Thus there seems to be no simple correlation between carcinogenicity and K- and L-region additivity toward ozone. It may be significant, however, that ozone does attack both carcinogens dibenz[a,h]anthracene (++)[57] and I[58] at those positions which are metabolically oxidized on elimination from test animals.

[57] P. M. Bhargava and C. Heidelberger, *J. Am. Chem. Soc.*, **78**, 3671 (1956).

[58] A. H. Conney, E. C. Miller and J. A. Miller, *J. Biol. Chem.*, **228**, 753 (1957).

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN, MADISON, WISC., AND OF STANFORD UNIVERSITY, STANFORD, CALIF.]

The Proton Magnetic Resonance Spectra of Some α-Acetoxy Ketones

BY KENNETH L. WILLIAMSON AND WILLIAM S. JOHNSON

RECEIVED JUNE 19, 1961

An examination of the n.m.r. spectra of six isomeric α-acetoxy ketones (2α-, 2β-, 4α- and 4β-acetoxycholestane-3-one and 3α- and 3β-acetoxycholestane-2-one) has revealed coupling constants for adjacent *cis* and *trans* protons (on tetrahedral carbons) larger than most of those previously reported, presumably because of the conformational rigidity of the A-ring. The use of larger parameters in the Karplus equation relating the dihedral angle between adjacent protons to the coupling constant has allowed the calculation of mutually consistent dihedral angles for these compounds from which it appears that 2β-acetoxycholestane-3-one exists in the twist conformation, a conclusion confirmed by optical rotatory dispersion measurements. In addition it has been found that the chemical shifts of equatorial protons are not always found downfield from their axial counterparts.

In order to apply nuclear magnetic resonance spectroscopy to the solution of stereochemical problems, two phenomena must be considered: namely, the field independent coupling constants and the field dependent chemical shifts of individual protons. From studies made on fourteen acetylated sugars and on two isomeric dimethoxycyclohexyl acetates, Lemieux, Kullnig and Moir[1] found that $J_{ae} = J_{ee} = 2$ to 3.5 c.p.s. and $J_{aa} = 5$ to 9 c.p.s. Karplus[2] has calculated the variation of the coupling constant of protons on adjacent carbon atoms as a function of the dihedral angle, ϕ, between these protons, and has obtained a reasonable correlation with the data of Lemieux, *et al.* The dihedral angles of the adjacent protons in these acetylated sugars were calculated on the assumption that the ring adopted a single chair conformation, a premise which may be open to question (see below). In all of these compounds the chemical shifts for equatorial protons were found at lower fields than were the shifts for axial protons.[3]

With this work in mind we examined the proton magnetic resonance spectra of six isomeric α-acetoxy ketones (2α-, 2β-, 4α- and 4β-acetoxycholestane-3-one and 3α- and 3β-acetoxycholestane-2-one) to gain some insight into the conformation of the A-ring in these compounds.

The resonance lines associated with the proton on the acetate-bearing carbon atom are displaced downfield from the other 47 protons in these keto-acetates because of the combined unshielding effects of both the adjacent carbonyl group and the acetate group. In 2α-acetoxycholestane-3-one (see Fig. 1),

[1] R. U. Lemieux, R. K. Kullnig and R. Y. Moir, *J. Am. Chem. Soc.*, **80**, 2237 (1958).

[2] M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

[3] (a) A. Novak and E. Whalley, *Can. J. Chem.*, **36**, 1116 (1958); (b) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).