Experimental Section

Infrared spectra were recorded in potassium bromide wafers using a Perkin-Elmer Model 237 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian A60A spectrophotometer. Melting points were taken on a Reichert polarizing hot stage. Elemental analyses were done by Galbraith Laboratories, Knoxville, Tenn. Mass spectra were taken by Morgan and Schaeffer, Montreal, Canada.

Phenylcinnamalone (I) was prepared as reported previously.[4]

Oxidation of Phenylcinnamalone (I) with Neutral Potassium Permanganate. Preparation of 6a′-Hydro-11a′-phenylspiro[isobenzofuran-3(1*H*),6′-benzo[*a*]fluorene]-1,5′,11′-trione (II). I (2 g) was dissolved in 200 mL of acetone. A saturated aqueous solution of potassium permanganate was added dropwise until the purple color persisted. The excess permanganate ion was destroyed with concentrated HCl. The manganese dioxide was removed by filtration, and the filtrate was evaporated until crystallization began. The solution was filtered after standing overnight. The collected solids were recrystallized from glacial acetic acid to yield 1.0 g (50%) of product: mp 270–272 °C; IR (cm^{-1})[12a] 3065 w, 2950 w, 1780 s, 1725 w, 1710 s, 1600 m, 1495 w, 1465 m, 1450 m, 1335 w, 1315 w, 1285 m, 1275 w, 1250 m, 1235 m, 1215 w, 1200 vw, 1190 w, 1165 w, 1130 w, 1105 m, 1075 vw, 1060 vw, 1035 m, 1000 m, 975 vw, 965 vw, 930 w, 925 m, 900 w, 895 w, 890 w, 875 vw, 830 w, 805 vw, 800 w, 780 m, 775 ms, 755 m, 750 m, 720 m 715 m, 700 m, 660 w, 650 w; ^{1}H NMR (Me_2SO-d_6, 120 °C) (δ)[12b] 5.12 (s, 1), 634 (m, 1), 7.50 (m, 16); mass spectrum, (*m/e*) 442 (P), 424, 414, 398, 397, 396, 386, 385, 252, 193, 165, 104, 77, 76. Isotopic analysis[12c] Calcd for $C_{30}H_{18}O_4$: P, 100; P + 1, 32.8; P + 2, 6.0. Found: P, 100; P + 1, 32.8; P + 2, 6.0. Anal. Calcd for $C_{30}H_{18}O_4$: C, 81.44; H, 4.09. Found: C, 81.10; H, 4.25.

Basic Hydrolysis of II. Formation of 6-(*o*-Carboxyphenyl)-11a-phenyl-5*H*-benzo[*a*]fluorene-5,10-dione (III). II (1 g) was dissolved in 20 mL of 1,4-dioxane. The solution was brought to reflux. 10% NaOH (20 mL) was added. The resultant mixture was refluxed for 4 h. Upon cooling, the solution was acidified with concentrated HCl. The precipitate was collected and crystallized from glacial acetic acid. The yield of product, mp 322–323 °C, was 0.7 g (70%): IR (cm^{-1}) 3060 w, 2900 s (broad), 1730 s, 1690 s, 1615 m, 1580 w, 1510 w, 1485 m, 1465 w, 1420 w, 1375 m, 1330 w, 1305 m, 1280 m, 1255 m, 1205 m, 1180 m, 1155 m, 1125 s, 1075 m, 1045 m, 975 w, 885 w, 875 w, 830 w, 810 w, 805 w, 785 w, 765 s, 740 m, 720 m, 700 w, 685 w, 675 w, 665 w, 640 w; ^{1}H NMR (Me_2SO-d_6, 37 °C) (δ) 7.40 (m, 15), 8.40 (m, 1), 8.68 (m, 1). Anal. Calcd for $C_{30}H_{18}O_4$: C, 81.44; H, 4.09. Found: C, 81.24; H, 3.94.

Registry No.—I, 18585-55-2; II, 69622-44-2; III, 69622-33-9.

References and Notes

(1) Research performed in part under the auspices of the Research Corporation.
(2) (a) New Mexico Institute of Mining and Technology; (b) Clarkson College of Technology; (c) Carnegie-Mellon University.
(3) Taken in part from the dissertations submitted by Drs. R. G. Brown, L. G. Donaruma, and R. A. Kropf in partial fulfillment of the requirements for a Ph.D. degree.
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(12) (a) s = strong absorption, m = medium absorption, w = weak absorption, and vw = very weak absorption. (b) First number is chemical shift; s = singlet and m = multiplet. (c) Isotopic analysis: P = parent peak, P + 1 = parent peak + 1, and P + 2 = parent peak + 2; numbers are relative intensities.

Radiation-Induced Oxidation of Benzo[*a*]pyrene[1]

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The oxidation in air of benzo[*a*]pyrene induced by ^{60}Co γ radiation gave over two dozen products, of which half have been identified. Products include three isomeric 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8,9,10-tetraols, 9,10-dihydrobenzo[*a*]pyrene-*trans*-9,10-diol, a 7,8-dihydrobenzo[*a*]pyrene-7,8-diol, a 4,5-dihydrobenzo[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-1,6-dione, benzo[*a*]pyrene-3,6-dione, benzo[*a*]pyrene-6,12-dione, 9-(2′formylphenyl)phenalen-1-one, and tentatively benzo[*a*]pyren-3-ol, benzo[*a*]pyren-6-ol, and benzo[*a*]pyren-9-ol. These results establish that air oxidation of benzo[*a*]pyrene yields products similar to those found as mammalian metabolites, with oxidative attack at the K region, bay region, and the 6 position. Some of the air oxidation products are weakly mutagenic toward *Salmonella typhimurium* test strains.

Mammalian metabolism of the common environmental pollutant benzo[*a*]pyrene (1) produces phenol, dihydrodiol, tetrahydrotetrol, epoxide, and dihydrodiol epoxide derivatives, some of which are powerful mutagens suspected of being proximate or ultimate carcinogens. Benzo[*a*]pyrene is also sensitive to radiation-induced air oxidation in solution,[3] as pure crystals,[4] and adsorbed on particulate matter.[4,5] Moreover, we have demonstrated that radiation-induced air oxidations of benzo[*a*]pyrene and of several other polycyclic aromatic hydrocarbons yield preparations mutagenic toward strains of *Salmonella typhimurium* without preliminary metabolic activation.[4]

We describe here the isolation and identification of oxidation products recovered from mutagenic preparations of oxidized benzo[*a*]pyrene and demonstrate thereby close similarities between air oxidation and oxidative metabolism of benzo[*a*]pyrene.

Results

Of the several irradiation conditions previously shown to transform benzo[*a*]pyrene into mutagenic material,[4] we chose for ease of product recovery the ^{60}Co γ irradiation of benzo[*a*]pyrene adsorbed on silica gel. Over two-dozen products were detected chromatographically, of which half have been isolated and identified by chromatographic and spectral data.

The major products were the well-known benzo[*a*]pyrene-1,6-, -3,6-, and -6,12-diones, **2**, **3**, and **4**, respectively.

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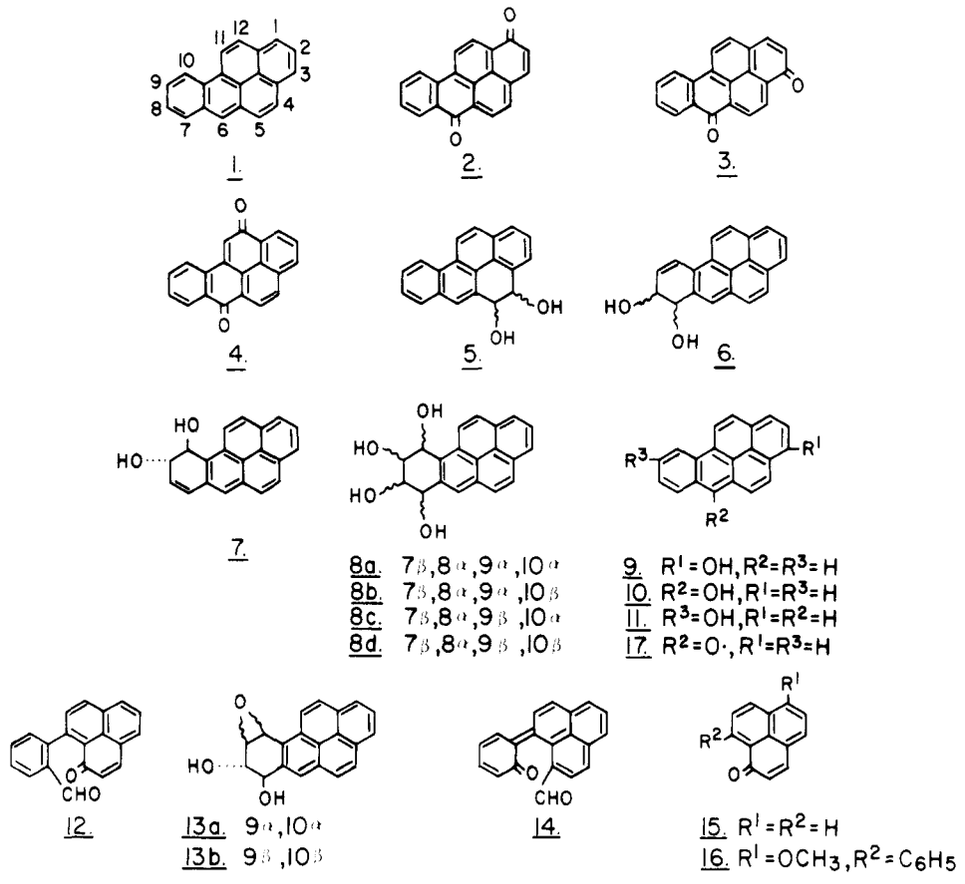

Minor products included the three dihydrodiols *rac*-4,5-dihydrobenzo[*a*]pyrene-4ξ,5ξ-diol (**5**), *rac*-7,8-dihydrobenzo[*a*]pyrene-7ξ,8ξ-diol (**6**), and *rac*-9,10-dihydrobenzo[*a*]pyrene-*trans*-9,10-diol (**7**), three isomeric *rac*-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8,9,10-tetraols (**8**), three benzo[*a*]pyrene phenols (**9–11**), and the novel secobenzo[*a*]pyrene derivative 9-(2′-formylphenyl)phenalen-1-one (**12**).

The first eluted products from reverse phase chromatography of oxidized benzo[*a*]pyrene preparations were three components recognized as tetrahydrotetraols by their retention times and mass spectra. Moreover, ultraviolet absorption spectra of each of these products were very similar to spectra of pyrene, 7,8,9,10-tetrahydrobenzo[*a*]pyrene, and 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8,9,10-tetraols derived metabolically or by hydrolysis of the isomeric *rac*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α-diols (**13**),[6] thus establishing these first-eluted components as isomeric 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8,9,10-tetraols (**8**). The initial tetraol corresponded in retention time with that of *rac*-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9β,10α-tetraol known to derive as minor product in the hydrolysis of the *rac*-9β,10β-epoxy-7β,8α-diol (**13b**). On this correspondence, identification of the component as *rac*-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9β,10α-tetraol (**8c**) is indicated. However, absorption at 282 nm in addition to the recognized pyrene-like spectrum of **8c** suggested the possible presence of an unrecognized impurity.

The second-eluted tetraol similarly corresponded in retention time to *rac*-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9α,10α-tetraol recognized as the minor tetraol product formed by hydrolysis of the *rac*-9α,10α-epoxy-7β,8α-diol (**13a**), and on this basis the product is identified as *rac*-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9α,10α-tetraol (**8a**). The third-eluted tetraol did not correspond in retention data with other known tetraols 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9α,10β-tetraol (**8b**) or 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7β,8α,9β,10β-tetraol (**8d**) and must accordingly be an undescribed 7,8,9,10-tetraol to which no stereochemistry can yet be assigned.[7]

The next eluted products included three dihydrodiols **5–7** recognized as such by their retention times and mass spectra. The most prominent product was recognized as being a 4,5-dihydro-4,5-diol **5** from its chrysene-like ultraviolet absorption spectrum which matched in detail published spectra of 4,5-dihydrobenzo[*a*]pyrene-*cis*-4,5-diol and of a 4,5-dihydrodiol metabolite as well. Although these data suffice for assignment of a 4,5-dihydro-4ξ,5ξ-diol structure to **5**, detailed stereochemistry cannot be assigned, and our preparation may be the *cis*-4,5-diol, *trans*-4,5-diol, or a mixture of both.

Likewise, retention data and ultraviolet absorption spectra of the next most prominent dihydrodiol were identical with those of authentic *rac*-7,8-dihydrobenzo[*a*]pyrene-*trans*-7,8-diol, and we assign a 7,8-dihydro-7,8-diol structure **6** to that component. Again, *cis*-7,8-diol, *trans*-7,8-diol, or a mixture of both may be implicated.

The third dihydrodiol of this group was identified more securely as the *trans*-9,10-diol **7**. Whereas retention time and ultraviolet absorption spectra evinced a 9,10-dihydro-9,10-diol structure, selection between cis and trans stereochemistry not possible with these data was achieved with proton nuclear magnetic resonance spectra. Proton spectra of our 9,10-diol **7** matched those of an authentic sample of *rac*-9,10-dihydrobenzo[*a*]pyrene-*trans*-9,10-diol and were different from reported details of proton spectra of the isomeric 9,10-dihydrobenzo[*a*]pyrene-*cis*-9,10-diol.[8]

It is assumed but not demonstrated that the product dihydrodiols **5–7** and tetrahydrotetraols **8** are all racemic.

A prominent oxidation product eluted with the dihydrodiols **5–7** was recognized as not being a previously identified benzo[*a*]pyrene metabolite or derivative by its spectral properties. A formulation $C_{20}H_{12}O_2$ established by mass spectra, carbonyl absorption at 1690 and 1640 cm^{-1}, and the absence of hydroxyl absorption bands and of active hydrogen strongly suggested a secodicarbonyl structure, derived in association

with carbon–carbon bond scission. Cleavage of any of the 24 carbon–carbon bonds was a priori possible, yielding putatively secodialadeydes with no or with one or two α-hydrogens (1,2, 2,3, 4,5, 7,8, 8,9, 9,10, and 11,12 scissions), secodiketones (10a,10b and interior bond scissions), ketoaldehydes with one α-hydrogen (3,3a, 3a,4, 5,5a, 6a,7, 10,10a, 10b,11, 12,12a, and 12a,1 scissions), as well as the two ketoaldehydes with no α-hydrogens derived by cleavage of the 5a,6 or 6,6a bonds.

Selection among these possibilities was reduced to two structures by proton spectra displaying a sharp, one-proton singlet at 9.76 ppm which was recognized as a signal from an aldehyde proton not coupled with adjacent α-hydrogen.[9] Only the 5a,6-seco-5a-ketone-6-aldehyde structure **12** or the alternative 6,6a-seco-6a-ketone-6-aldehyde structure **14** is consistent with this datum. Choice between structures **12** and **14** was made from additional consideration of proton and ultraviolet absorption spectra.

Proton spectra resemble in detail those of several model compounds, including phenalen-1-one (**15**) and 6-methoxy-9-phenylphenalen-1-one (**16**).[10] Of particular importance is the uniform presence of a characteristic AX pattern for the H-2 and H-3 protons of the seco product, **15**, and of **16**, all with identical $J_{2,3}$ = 9.5 Hz. Proton spectra of the alternative structure **14** should reveal the vinyl proton adjacent to the ketone carbonyl group as the X moiety of an ABX or more complex pattern.

Moreover, the ultraviolet absorption spectrum of the secobenzo[*a*]pyrene product resembles in detail spectra of **15**. Absorption spectra of the alternative structure **14** with the double bond exocyclic to both ring moieties would be expected to be more markedly different. From these arguments we assign the structure 9-(2′-formylphenyl)phenalen-1-one or 5a-oxo-5a,6-secobenzo[*a*]pyrene-6-al (**12**) to this oxidation product in preference to the alternative 6,6a-seco structure **14**.

Chromatographic and fluorescence excitation spectral evidence was also obtained for the presence of three very minor products considered to be benzo[*a*]pyrene phenols. One component with the same retention time as benzo[*a*]pyren-9-ol (**11**) gave spectra very similar to but not identical with those of the authentic 9-phenol **11**. A second chromatographic component with retention time of benzo[*a*]pyren-3-ol (**9**) and benzo[*a*]pyren-6-ol (**10**) exhibited fluorescence excitation spectra which appeared to be a composite of spectra from the 3-phenol **9** and 6-phenol **10**. We tentatively assign the 3-, 6-, and 9-phenol structures **9**–**11** to these components accordingly.

Discussion

The same kinds of benzo[*a*]pyrene oxidation products implicated in mammalian metabolism are obtained in these radiation-induced air oxidations. Thus, the well-recognized 4,5-, 7,8-, and 9,10-dihydrodiol metabolites are likewise produced by air oxidation. Moreover, three isomeric 7,8,9,10-tetrahydrotetraols (**8**) were formed, two of which (**8a, 8c**) are recognized metabolities. The three phenols **9**–**11** and three quinones **2**–**4** are also variously implicated in benzo[*a*]pyrene metabolism.

Only the seco product **12** is unique to our air-oxidation conditions, no secobenzo[*a*]pyrene metabolite having been detected thus far. However, a proposal linking putative electronically excited secobenzo[*a*]pyrene dicarbonyl metabolites and chemiluminescence associated with benzo[*a*]pyrene metabolism has been made.[11] Our discovery of the seco derivative **12** formed nonenzymically may stimulate search for secobenzo[*a*]pyrene metabolites.

Our results clearly demonstrate the sensitivity of benzo[*a*]pyrene to air oxidations in the K region, bay region, the 7,8 positions, and the 6 position. The sensitivity of benzo[*a*]pyrene to chemical[12] and electrochemical[13] oxidations in the 6 position is well known, with initial formation of the benzo[*a*]pyrene-6-radical cation and benzo[*a*]pyrene-6-oxyl radical (**17**), leading ultimately to the formation of the 6-phenol **10** and the quinones **2**–**4**.[13,14] Benzo[*a*]pyrene forms at least four different radicals under different conditions, one being the radical cation and one the 6-oxyl radical **17**.[15]

The 5a,6-seco product **12** is also clearly a product of oxidative attack at the 6 position, possibly via the same 6-oxyl radical **17**. As reduced dioxygen species (hydrogen peroxide, superoxide) and hydroxyl radical as well as molecular oxygen and water may be implicated in the air oxidation of benzo[*a*]pyrene to products **2**–**4**,[15] further speculation on the mechanism of derivation of **12** will not be attempted here.

Although the same dihydrodiols **5**–**7** appear to be formed metabolically as well as by air oxidations, it is not certain that the same reaction mechanisms be implicated in both cases. The dihydrodiol metabolites are generally considered to arise via initial mixed function oxidase oxidation of benzo[*a*]pyrene to an intermediate dihydro epoxide which upon spontaneous or enzymic hydration yields the corresponding dihydrodiol. However, the microbial oxidation or benzo[*a*]pyrene to a 9,10-dihydro-*cis*-9,10-diol metabolite[8] suggests diverse metabolic processes occur which may not follow the same transformation pathway.

Dihydrodiol formation in the case of the *trans*-9,10-diol **7** obtained in our work may conform to preferential trans hydration of a putative 9,10-epoxide precursor, but evidence for the presence of such an epoxide could not be adduced. In the case of the 4,5- and 7,8-diols **5** and **6**, as the stereochemistry is unknown, we cannot comment further on possible mechanisms. However, cis diols if they occur might derive from intermediate dioxetane derivatives or by mechanisms involving sequential attack of more than one oxygen molecule, etc.

The oxidized benzo[*a*]pyrene preparations in which we found products **2**–**12** were weakly mutagenic toward *Salmonella typhimurium* strain TA 98,[4] and we have achieved some concentration of mutagens into several fractions resolved chromatographically from these preparations.[16] However, it is apparent that the quinones **2**–**4** and dihydrodiols **5**–**7** implicated in our studies do not account for the mutagenicity of these oxidized benzo[*a*]pyrene samples, as none of the products **2**–**7** is mutagenic toward *S. typhimurium*.[17] Different preparations of the seco derivative **12** consistently showed weak dose response mutagenicity toward *S. typhimurium* strain TA 98,[18] but **12** cannot account for the mutagenic responses found in other chromatographic fractions not containing **12**. Accordingly, yet other undetected potent mutagenic species must be present in these preparations.[19] The 3-phenol **9** and 6-phenol **10** probably present in our preparations are weakly and moderately mutagenic respectively toward *S. typhimurium* strain TA 98[17] and may account for some of the mutagenicity found.

Although we did not design these experiments to simulate environmental conditions, our results may still have some significance in this regard. We have observed mutagenic responses in oxidized benzo[*a*]pyrene preparations, whether ionizing radiation or visible or ultraviolet light be used to initiate oxidations, and very preliminary chromatographic examination of preparations made without ionizing radiation suggest that some of the same products (**2**–**4**) are formed. We may speculate that other products **5**–**12** may also be formed in oxidations involving ultraviolet or visible light.

As benzo[*a*]pyrene is well distributed in the biosphere and exposed to air and solar radiation, the possibilities that these processes transform benzo[*a*]pyrene into mutagenic (or carcinogenic) derivatives need attention. In this regard, frameshift mutagenicity toward strains of *S. typhimurium* of air-

borne particulate matter has been repeatedly demonstrated.[20,21]

Experimental Section

Benzo[a]pyrene (99+% Gold Label) and phenalen-1-one were from Aldrich Chemical Co., Milwaukee, Wis. Benzo[a]pyrene was analyzed by chromatography and mass spectrometry and shown to be free from detectable impurities before use. Reagent grade silica gel (60–200 mesh) from J. T. Baker Chemical Co., Philipsburg, N.J., was washed with benzene–methanol (4:1) before use.

Thin-layer chromatography was conducted with 20 × 20 cm chromatoplates of Silica Gel HF_{254} (E. Merck GmbH., Darmstadt) (0.25 mm thick for analyses, 1.0 mm thick for preparative operations) irrigated in ascending fashion with benzene or benzene–ethanol (19:1). High-performance liquid column chromatography was conducted using Waters Associates (Milford, Mass.) Model 6000A pumps and Model 660 solvent programmer accessory with effluent monitoring with a Perkin-Elmer Corp. Model LC55 variable wavelength spectrophotometer set at 250 nm. Two reverse-phase systems employing aqueous methanol as mobile phase were used: system 1, a 100 cm × 0.4 cm i.d., ODS Permaphase column (DuPont Inc.) operated with a linear program from 30% methanol to 70% methanol over 50 min at a flow rate of 0.8 mL/min; system 2, a 30 cm × 0.4 cm i.d. μ-Bondapak-C_{18} column (Waters Associates) operated with a linear program from 30% methanol to 70% methanol over 80 min at a flow rate of 1.0 mL/min.

Ultraviolet spectra were recorded on methanol solutions using a Cary Model 14 spectrophotometer. Infrared spectra were recorded over the range 400–4000 cm^{-1} using a Perkin-Elmer Model 337 spectrophotometer equipped with beam condenser and samples incorporated into 1-mm diameter KBr disks. Mass spectra were obtained using a Finnigan Corp. Model 3200 quadrupole mass spectrometer equipped for both EI and CI mass spectrometry. Methane and ammonia were used as reagent gases in CI operations. Samples were introduced as solids by direct probe. High-resolution mass spectra were obtained with a CEC 21-110B mass spectrometer. Fourier transform proton spectra were recorded using a Varian Model XL-100 spectrometer equipped with a deuterium lock using perdeuterioacetone solutions of samples also containing an internal reference of tetramethylsilane. Chemical shifts are measured downfield from the internal reference.

Mutagenicity assays were conducted with *S. typhimurium* strain TA 98 by means which we have previously described in detail.[4]

Benzo[a]pyrene samples (60–400 mg) dissolved in benzene were adsorbed onto a 100-fold excesses of silica gel, and after solvent removal under vacuum the material was irradiated in air with ^{60}Co γ radiation in a Gammacell 200 (Atomic Energy of Canada Ltd., Ottawa) for 36 h (1.4×10^5 rad/h). Products were recovered by extracting the silica gel with three 25-mL portions of benzene–methanol (4:1) and evaporating the extracts under vacuum, thereby obtaining a brown gum. Following thin-layer chromatography of the mixture using benzene–ethanol (19:1) to remove most unaltered benzo[a]pyrene, the oxidized products were eluted with benzene–ethanol (4:1) and recovered after solvent removal under vacuum for reverse-phase column chromatography.

Isolation of Products. High-performance liquid column chromatography of reaction products in system 2 resolved 24 components characterized by t_R: No. 1, 45 min; No. 2, 47.5 min; No. 3, 49 min; No. 4, 51 min; No. 5, 54 min; No. 6, 58 min; No. 7, 60 min; No. 8, 62.5 min; No. 9, 67 min; No. 10, 68 min; No. 11, 69 min; No. 12, 71 min; No. 13, 74 min; No. 14, 78 min; No. 15, 79 min; No. 16, 81 min; No. 17, 82.5 min; No. 18, 84 min; No. 19, 86 min; No. 20, 87 min; No. 21, 90 min; No. 22, 109 min; No. 23, 115 min; No. 24, 119 min. Repeated oxidations and analyses confirmed this pattern. Individual fractions were collected, evaporated under vacuum, and analyzed further as described. From a typical experiment involving irradiation of 304 mg of benzo[a]pyrene the following data were collected.

***rac*-7,8,9,10-Tetrahydrobenzo[*a*]pyrene-7β,8α,9β,10α-tetraol (8c).** The first eluted component No. 1 recovered as 84 μg (0.02%) of colorless amorphous solids had the same t_R (45 min) as the 7β,8α,9β,10α-tetraol **8c** derived by hydrolysis of *rac*-9β,10β-epoxy-7β,8α-diol **13b** and was identified further by ultraviolet absorption spectra.

***rac*-7,8,9,10-Tetrahydrobenzo[*a*]pyrene-7β,8α,9α,10α-tetraol (8a).** Component No. 2 recovered as 33 μg (0.01%) of colorless glass had the same t_R (47.5 min) as the 7β,8α,9α,10α-tetraol **8a** derived by hydrolysis of the *rac*-9α,10α-epoxy-7β,8α-diol **13a** and was identified further by ultraviolet absorption spectra and by mass spectra.

***rac*-7,8,9,10-Tetrahydrobenzo[*a*]pyrene-7ξ,8ξ,9ξ,10ξ-tetraol (8).** Component No. 3 with t_R 49 min recovered as a yellow glass (35 μg, 0.01%) was recognized as a tetrahydrotetraol by ultraviolet absorption spectra and by mass spectra. The t_R of component No. 3 did not correspond with those of any of the four tetraols derived by hydrolysis of the 9,10-epoxy-7β,8α-diols **13**, vide infra.[6]

Isomeric 7,8,9,10-Tetrahydrobenzo[*a*]pyrene-7β,8α,9,10-tetraols, (8a–d). A solution of 1.0 mg each of *rac*-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7β,8α-diol (**13a**) and *rac*-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7β,8α-diol (**13b**) in 200 mL of tetrahydrofuran–water (1:4) was acidified to pH 3 with dilute HCl and stirred at room temperature for 12 h. Products were extracted with three 10-mL portions of benzene–ethyl acetate (4:1); the extracts were evaporated under vacuum and chromatographed by reverse-phase methods in system 2. Material eluted between 42 and 53 min was recovered and evaporated under vacuum to yield the tetraols **8a–d** mixture as colorless amorphous solids recognized by ultraviolet absorption spectra and by mass spectra as containing tetrahydrotetraol derivatives. Reverse-phase chromatography in system 2 gave the characteristic four-component pattern of 42.5 (**8b**), 45 (**8c**), 47.5 (**8a**), and 52.5 min (**8d**).[6]

***rac*-9,10-Dihydrobenzo[*a*]pyrene-*trans*-9,10-diol (7).** Component No. 4 yielded 103 μg (0.03%) of colorless glass with ultraviolet, ^1H NMR, and mass spectra and t_R (51 min) identical with those of an authentic sample of the *trans*-9,10-diol **7**.

***rac*-4,5-Dihydrobenzo[*a*]pyrene-4ξ,5ξ-diol (5).** Component No. 12 with t_R 71 min recovered as 195 μg (0.06%) as a glass was recognized as being a 4,5-dihydrodiol **5** by ultraviolet absorption spectra, mass spectra, and t_R.

***rac*-7,8-Dihydrobenzo[*a*]pyrene-7ξ,8ξ-diol (6).** Component No. 13 with t_R 74 min recovered as 140 μg (0.04%) of colorless amorphous solid was recognized as being a 7,8-dihydrodiol **6** by ultraviolet absorption spectra, mass spectra, and t_R.

9-(2′-Formylphenyl)phenalen-1-one (12). Component No. 8 with t_R 62.5 min gave 95 μg (0.03%) of a yellow amorphous glass: λ_{max} (MeOH) (ε) 245 (24 500), 276 (8250), 295 (3000), 309 (2250), 325 (1750), 342 (5250), 361 (6500), 387 nm (2000); $\tilde{\nu}_{max}$ (KBr) 1690 (CHO), 1640 cm^{-1} (CO); CI mass spectrum (CH_4) *m/z* 313 (20) $(M + C_2H_5)^+$, 285 (100) $(M + 1)^+$, 255 (8) $(M - CHO)^+$; CI mass spectrum (NH_3) 285 (100) $(M + 1)^+$ and (N^2H_3) 286 (100); ^1H NMR spectrum δ 6.47 (1 H, d, $J_{2,3}$ = 9.5 Hz, H-2), 7.30 (3 H, unresolved, H-4′, H-5′, H-6′), 7.62 (1 H, d, $J_{7,8}$ = 8 Hz, H-8), 7.60–7.88 (3 H, unresolved, H-4, H-5, H-3′), 7.93 (1 H, d, $J_{2,3}$ = 9.5 Hz, H-3), 8.28 (1 H, d of d, $J_{5,6}$ = 8 Hz, $J_{4,6}$ = 1.5 Hz, H-6), 8.44 (1 H, d, $J_{7,8}$ = 8 Hz, H-7), 9.76 (1 H, s, CHO).

Anal. Calcd for $C_{20}H_{20}O_2$: M, 284.0837. Found: M, 284.0790.

Unidentified Oxidation Products. Component No. 15 with t_R 79 min recovered as 164 μg of an orange amorphous solid was recognized as being a benzo[a]pyrene oxidation product with apparent M 290 from mass spectra.

Benzo[*a*]pyrene-1,6-dione (2). Component No. 16 with t_R 81 min was recognized as the 1,6-dione **2** by comparison of chromatographic and spectral properties with the reference sample. The 1,6-dione **2** was also isolated from a larger (60 mg) sample of irradiated benzo[a]pyrene. Thin-layer chromatography in benzene yielded 5 mg of mixed diones **2**, **3**, and **4** which were resolved using reverse-phase column chromatography in system 1. The first eluted component at t_R 43 min gave 0.80 mg (1.2%) of gold colored solids, mp (Kofler) 289–290 °C (lit. mp 287–288 °C[14a] and 293 °C;[22] mp (Kofler) authentic **2**, 291–295 °C), identified as the 1,6-dione **2** by additional chromatographic and spectral data.

Benzo[*a*]pyrene-3,6-dione (3). The second dione eluted at t_R 45 min (correlated with component No. 17 with t_R 82.5 min in system 2) was recovered as 0.65 mg (1.0%) of red crystals, mp (Kofler) 287–290 °C (lit. mp 288–289[14a] and 290–291 °C[22]), identified as the 3,6-dione **3** by additional chromatographic and spectral data.

Benzo[*a*]pyrene-6,12-dione (4). The third quinone eluted at t_R 47 min (correlated with component No. 18 with t_R 84 min in system 2) was obtained as 0.55 mg (1.0%) of orange crystals, mp (Kofler) 321–322 °C (lit. mp 320–321[14a] and 326 °C[22]), identified as the 6,12-dione **4** by additional chromatographic and spectral data.

Benzo[*a*]pyrene (1). Component No. 23 with t_R 115 min (t_R 80 min in system 1) was identified as unreacted benzo[a]pyrene by additional chromatographic and spectral data, in comparison with data from authentic material.

Benzo[*a*]pyrene Phenols. Very minor components eluted after the diones **2–4** but before benzo[a]pyrene included several phenols which were examined by alternative methods. Oxidized benzo[a]pyrene was subjected to thin-layer chromatography using benzene, and material eluted from the region R_f 0–0.55 was then subjected to reverse-phase chromatography using system 1 but with a shorter

program time. Extremely small amounts of two components were recovered. The first component eluted had t_R 47 min identical with that of authentic **11** and fluorescence excitation spectra in 1% methanolic NaOH similar to that of **11**. The second component eluted had t_R 55 min identical with those of authentic **9** and **10** and fluorescence excitation spectra with features of spectra of both **9** and **10**.

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Registry No.—**1**, 50-32-8; **2**, 3067-13-8; **3**, 3067-14-9; **4**, 3067-12-7; **5**, 28622-84-6; **6**, 13345-25-0; **7**, 58030-91-4; **8a**, 62697-16-9; **8b**, 62697-19-2; **8c**, 62697-17-0; **8d**, 62697-13-6; **9**, 13345-21-6; **10**, 33953-73-0; **11**, 17573-21-6; **12**, 55669-61-9; **13a**, 58917-67-2; **13b**, 58917-91-2.

Supplementary Material Available: A detailed comparison of spectra of **12** with those of **15** and **16** as well as full spectral characterization of identified benzo[*a*]pyrene derivatives **2–11** (7 pages). Ordering information is given on any current masthead page.

References and Notes

(1) A preliminary account of some of these results has been presented: cf. (a) T. L. Gibson, V. B. Smart, and L. L. Smith, Abstracts of Papers, 32nd Southwest Regional Meetings of the American Chemical Society, Ft. Worth, Texas, Dec. 1–3, 1976, p 24; (b) T. L. Gibson, V. B. Smart, and L. L. Smith, Abstracts of Papers, 173rd National Meeting of the American Chemical Society, New Orleans, La., March 20–25, 1977, ENVT-113; *Prepr., Div. Environmental Chem.*, **17**, 327 (1977).

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(6) Hydrolysis of *rac*-**13a** gave *rac*-**8a** and *rac*-**8b**; hydrolysis of *rac*-**13b** gave *rac*-**8c** and *rac*-**8d**: cf. (a) E. Huberman, L. Sachs, S. K. Yang, and H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 607 (1976); (b) S. K. Yang, D. W. McCourt, F. P. Roller, and H. V. Gelboin, *ibid.*, **73**, 2594 (1976); (c) S. K. Yang and H. V. Gelboin, *Biochem. Pharmacol.*, **25**, 2221, i (1976); (d) D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conner, and D. M. Jerina, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3381 (1976); (e) S. K. Yang, D. W. McCourt, H. V. Gelboin, J. R. Miller, and P. P. Roller, *J. Am. Chem. Soc.*, **99**, 5124 (1977); (f) S. K. Yang, D. W. McCourt, and H. V. Gelboin, *ibid.*, **99**, 5130 (1977).

(7) Four isomeric tetraols with cis oriented 7,8-diol features not heretofore described in the literature are theoretically possible.

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(9) A related synthetic secodialdehyde 4,5-secobenzo[*a*]pyrene-4,5-dial exhibits two singlet aldehyde proton signals at δ 10.05 and 10.88: cf. R. G. Harvey, S. H. Goh, and C. Cortez, *J. Am. Chem. Soc.*, **97**, 3468 (1975).

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(15) The benzo[*a*]pyrene-6-radical cation is observed in strong acid solutions of benzo[*a*]pyrene; the 6-oxyl radical **17** is formed under a variety of conditions involving the oxidation of benzo[*a*]pyrene or the 6-phenol **10**; a benzo[*a*]pyrene radical anion is formed by alkali metal reduction of benzo[*a*]pyrene; a fourth radical formed by heating benzo[*a*]pyrene may represent rearrangement to azuleno[1,2,3-*cd*]phenalene, cf. E. M. Menger, R. B. Spokane, and P. D. Sullivan, *Biochem. Biophys. Res. Commun.*, **71**, 610 (1976), and references cited therein.

(16) Mutagenic responses are weak but are reproducible, and a dose response relationship is shown. We consider an oxidized polycyclic aromatic hydrocarbon to be mutagenic when the number of bacterial revertants in a test is three times that of the control, that is, that a twofold increase in revertants over the spontaneous rate be had. Oxidized benzo[*a*]pyrene preparations show a dose response mutagenicity toward *S. typhimurium* strain TA 98, a twofold increase in revertants typically being achieved with about 100 μg of oxidized benzo[*a*]pyrene.[4] Mutagenicity was distributed among the several oxidized components resolved by system 2. Early eluted products (t_R 0–55 min) including the tetraols **8** and 9,10-diol **7** showed dose response over the range 5–20 μg, with a twofold increase in revertants with approximately 6 μg of mixed products. The material eluted next (t_R 55–82 min) including **12** and dihydrodiol **5** and **6** likewise exhibited dose response over the range 10–50 μg, with twofold increase in revertants with approximately 27 μg. A third fraction (t_R 82–90 min) containing the quinones **2–4** and possibly phenols gave a constant eightfold increase in revertants but no dose response over the 30–200-μg range tested. A final fraction (t_R 90–125 min) containing recovered benzo[*a*]pyrene likewise gave a constant sevenfold increase in revertants but no dose response over the 25–100-μg range tested.

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(18) A dose response over the range 8–46 μg was indicated, with twofold increase in revertants obtained with approximately 20 μg **12**.

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