

CURRENT RESEARCH

Chromatographic Isolation and Spectral Analysis of Polycyclic Quinones Application to Air Pollution Analysis

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■ A rapid and simple method is described for the analysis of polycyclic quinones (PQu's) derived from polynuclear aromatic hydrocarbons (PAH's), involving resolution by column and thin-layer chromatography and spectral analysis by ultraviolet, visible and fluorescence spectrophotometry, and mass spectrometry. Isomeric PQu's were isolated on silica gel columns and polyamide or magnesium hydroxide thin-layer plates. Applications of the above technique to photooxidation and air pollution studies are reported. 9,10-Anthraquinone, benzo[a]pyrene-6,12-quinone, benzo[a]pyrene-1,6-quinone, benzo[a]pyrene-3,6-quinone, and dibenzo[b,def]chrysene-7,14-quinone were identified in samples of suspended particulate matter collected in Toronto, Ont. Because of the very low concentrations of PQu's in the atmosphere, the collected quinones were reduced to the parent hydrocarbon prior to fluorescence analysis so that quantitative analyses could be performed. Estimations are reported of the monthly distribution in the amount of benzene soluble fraction, quinone fraction, benzo[a]pyrene, benz[a]anthracene, benzo[a]pyrene-quinone, benz[a]anthracene-quinone, and benzanthrone in suspended particulate matter, collected as part of a one-year study at York University, Toronto, Ont. The ratios of PQu content to the parent hydrocarbon content are correlated with ease of photooxidation and seasonal variations in fuel-burning practices.

Polynuclear aromatic hydrocarbons (PAH's) are known to be widely distributed in the atmospheric environment. Some of these compounds have proved to be carcinogenic for experimental animals and have been associated with the incidence of various types of cancer in man. Although much information has been obtained on the isolation and identification of these compounds, very little information has been amassed on the polycyclic quinones (PQu's) derived from these hydrocarbons.

Polycyclic quinones may be formed from the appropriate PAH's by their photochemical oxidation in the atmosphere (1, 2). There is evidence that PAH's are degraded in the atmosphere by photooxidation, by reaction with atmospheric oxidants, or by reaction with sulfur oxides (3). The most likely reactions of these hydrocarbons produce oxygenated compounds. Some oxygenated compounds exist in the atmosphere, and the oxygenated fractions of several air extracts appear to be carcinogenic (4, 5). Several investigations (6, 7) have indicated that various PAH's are metabolized in mammalian cells with some of the end products being polycyclic quinones.

The limiting factor in the above studies seems to be the lack of a relatively good separation system for the analysis of the polycyclic quinones. Column chromatography has been attempted in the resolution of isomeric quinones derived from pyrene (8) and benzo[a]pyrene (9), while thin-

layer chromatography has attempted to separate the quinones of benzo[a]pyrene (10, 11) and perylene (12). However, none of these methods appear to be sufficiently sensitive for the isolation of polycyclic quinones of the same molecular weight.

The separation of 10 polycyclic quinones, ranging in molecular weight from 208–332, has been studied using column and thin-layer chromatography. Spectral data, including ultraviolet absorption band positions and intensities, together with mass spectral data, were also recorded. Applications to photooxidation and air pollution studies are discussed.

Experimental

The chemical names of the PQu's were derived from the Ring Index (13) and, together with numerical designation, molecular formulas, and melting points, appear in Table I. Structures of the PQu's investigated in this study are illustrated in Figure 1.

Preparation of Standard Polycyclic Quinones. 9,10-Anthraquinone and dibenzo[b,def]chrysene-7,14-quinone were obtained from commercial sources and recrystallized to constant melting point. Purity was then ascertained by thin-layer chromatography. Because the commercial stock of PQu's is limited to the above two compounds, other PQu's were synthesized using mg quantities of polynuclear aromatic hydrocarbons in large-scale preparative oxidation reactions.

Pyrene-1,6-quinone and pyrene-1,8-quinone were prepared by the chromic acid oxidation of pyrene (9, 14). Benzo[a]anthracene-7,12-quinone and three quinones of benzo[a]pyrene—i.e., benzo[a]pyrene-6,12-quinone, benzo[a]pyrene-1,6-quinone, and benzo[a]pyrene-3,6-quinone were also prepared by chromic acid oxidation of benz[a]-anthracene and benzo[a]pyrene, respectively. Benz[a]-

Table I. Polycyclic Quinones Derived from Polynuclear Aromatic Hydrocarbons

Compound no.	Compound name	Molecular formula	Uncorrected m.p., °C
I	9,10-Anthraquinone	C ₁₄ H ₈ O ₂	283
II	Pyrene-1,6-quinone	C ₁₆ H ₈ O ₂	310
III	Pyrene-1,8-quinone	C ₁₆ H ₈ O ₂	271
IV	Benz[a]anthracene-7,12-quinone	C ₁₈ H ₁₀ O ₂	168
V	Benz[a]anthracene-5,6-quinone	C ₁₈ H ₁₀ O ₂	262
VI	Benzo[a]pyrene-6,12-quinone	C ₂₀ H ₁₀ O ₂	327
VII	Benzo[a]pyrene-1,6-quinone	C ₂₀ H ₁₀ O ₂	291
VIII	Benzo[a]pyrene-3,6-quinone	C ₂₀ H ₁₀ O ₂	292
IX	Benzo[a]pyrene-4,5-quinone	C ₂₀ H ₁₀ O ₂	258
X	Dibenzo[b,def]chrysene-5,6-quinone	C ₂₄ H ₁₂ O ₂	390

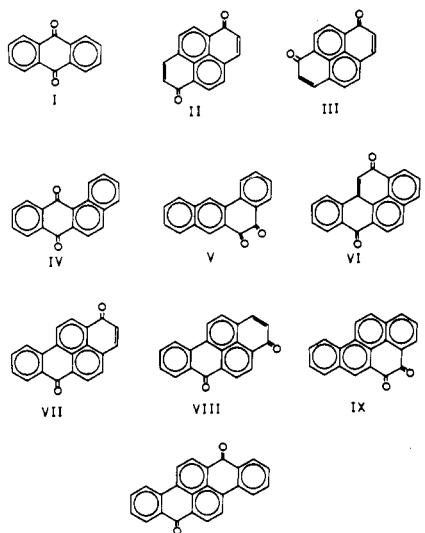


Figure 1. Chemical structures of PQu's derived from polycyclic arenes

anthracene-5,6-quinone and benzo[*a*]pyrene-4,5-quinone were prepared by the osmium tetroxide oxidation of benz[*a*]anthracene and benzo[*a*]pyrene, respectively (15, 16).

Microgram quantities of pure benzo[*a*]pyrene (BaP) and benz[*a*]anthracene (BaA) were photooxidized in a simulated atmosphere of 2-ppm ozone and a radiation source similar to solar radiation in the region of 2900–5000 Å corresponding to a solar zenith angle of $Z = 40^\circ$ to $Z = 60^\circ$ (17). In addition to recording the rate of photooxidation, some of the products were recovered and subjected to thin-layer chromatography.

Preliminary purification of the synthesized quinone mixtures was carried out using column chromatography on silica gel (Merck, 70–230 mesh, activated at 140°C for 24 hr). The column was prepared by slowly adding a slurry of silica gel in ice-cooled chloroform to a clean glass column (30 × 1 cm), previously rinsed and half filled with CHCl₃, to an adsorbent height of 20 cm. The quinone to be isolated was dissolved in 0.1–0.5 ml CHCl₃ and placed at the top of the column. A flow rate of 1.5 ml/min was maintained throughout with CHCl₃ as the eluting agent. Any unreacted hydrocarbon was collected in the first 30–40 ml, while the quinone fraction was collected in a 10-ml fraction after 70–80 ml of eluate. Final isolation of the isomeric quinones was achieved with preparative thin-layer chromatography on Mg(OH)₂ (14) using either CHCl₃ or CH₂Cl₂ as the mobile phase. Identification of the isolated quinones was accomplished by comparing the ultraviolet and mass spectra of the synthesized quinones with spectra of model PQu's. Purity was determined by comparison of experimental melting points with literature values (14–16).

The above PQu's were then used in the elucidation of an appropriate separation and identification procedure to be employed in the analysis of PQu's present in suspended particulate matter extracts.

Thin-Layer Chromatography and Spectral Analysis. Standard solutions of polycyclic quinones (2–5 µg/spot) were applied to a Polyamide 11 thin-layer plate (Brinkmann) and developed in acetic acid:water (19:1, v/v). Adsorption chromatography on thin layers of Mg(OH)₂ with various chlorinated solvents as the mobile phases, was used for both preparative and analytical separations. Parent polycyclic arenes were separated on aluminum oxide thin-layer plates with hexane:ether (19:1, v/v) as the developing phase. Benzanthrone (7H-benz[*d*e]anthracen-7-one) was resolved on aluminum oxide thin-layer plates using pen-

tane:chloroform (3:2, v/v) as the mobile phase (18). All thin-layer chromatography was carried out in sandwich chambers (19) (Brinkmann, Model 116C).

Ultraviolet and visible spectra of each quinone were recorded on a Cary 14 ratio recording spectrophotometer. Spectrograde CHCl₃ was used as the solvent. Luminescence analysis was carried out on a Farrand MK I spectrofluorometer using spectrograde pentane or hexane as the solvent. Concentrated sulfuric acid was used as the solvent for the luminescence analysis of benzanthrone. Mass spectra were recorded at an ionizing voltage of 70 eV employing a Perkin-Elmer-Hitachi RMU 6E mass spectrometer.

Application to Air Pollution Analysis. Monthly composites of benzene soluble fractions (BSF's) obtained from the Soxhlet extraction of suspended particulate matter collected in a northern, suburban location in Toronto, Ont. (20, 21), were carefully weighed and divided after evaporation under a stream of dry nitrogen at room temperature. A weighed portion of each residue was then dissolved in 250–500 µl CHCl₃ and an aliquot of each chloroform extract was subjected to column chromatography on silica gel, as described previously. The polycyclic quinone fraction (PQF) was collected and subjected to gravimetric analysis after drying. The PQF may be defined as that fraction having the same retention time as does a synthetic mixture of polycyclic quinones eluted from an identical column. Aliquots of the PQF, along with the appropriate synthetic PQu's to act as internal standards, were applied to polyamide thin-layer plates and developed in acetic acid:water (19:1, v/v). Upon completion of development and air drying, the chromatogram was viewed under long-wavelength ultraviolet light and the appropriate spots were scored. The spots were removed from the plate and eluted for 30 min in anhydrous diethyl ether (20–30 ml) in 50-ml weighing bottles equipped with ground glass lids. On completion of extraction in the dark at room temperature, the eluate was filtered and evaporated under dry nitrogen. The residue was taken up in spectrograde chloroform, ultraviolet spectra were recorded at room temperature, and the resultant spectra compared to the model spectra of synthesized PQu's.

Due to the small concentration of PQu's in the BSF's, in addition to the relative insensitivity of ultraviolet absorption measurements compared to fluorescence measurements (3), the recovered PQu's present in the PQF's were reduced to their corresponding polycyclic aromatic hydrocarbons. The resultant highly fluorescent compounds could then be quantitated using fluorescence spectrophotometry.

Standard PQu's, in milligram quantities, were reduced to their parent aromatic hydrocarbons with aluminum tricyclohexyl oxide in cyclohexanol (22). Reduction for 48 hr with subsequent removal of the cyclohexanol by distillation yielded the crude reduction mixture. The PAH's were extracted with diethyl ether and recrystallized to constant melting point. Ultraviolet and fluorescence spectra were then recorded. Once the above preliminary reduction reactions were shown to be successful, the contents of starting materials were scaled down to microgram quantities of PQu's. Subsequent to reduction, the PAH's were extracted, but because of the small amounts of starting materials, the final products were not crystallized but were allowed to remain as oils. These oils were then chromatographed on alumina thin-layer plates, the PAH's eluted and quantitated by fluorescence spectrophotometry. The PQF's, collected from the BSF's were reduced and subjected to the same thin-layer chromatographic isolation and fluorescence quantitative analysis as the above microgram-scale reduction mixtures. The recoveries from the reduction of the microgram quantities of standard PQu's, in addition to the

Table II. R_f Values of Polycyclic Quinones Separated by Thin-Layer Chromatography (TLC layer + developing phase)^a

Compound no.	Polyamide HOAc:H ₂ O (19:1)	Mg(OH) ₂ CHCl ₃	Mg(OH) ₂ CH ₂ Cl ₂
I	0.71	0.82	0.99
II	0.67	0.71	0.88
III	0.66	0.65	0.77
IV	0.59	0.72	0.75
V	0.48	0.41	0.56
VI	0.47	0.47	0.56
VII	0.45	0.41	0.59
VIII	0.40	0.35	0.52
IX	0.38	0.43	0.49
X	0.14	0.25	0.33

^a Geometric mean of multiple determinations.

Table III. Ultraviolet and Visible Spectra of Polycyclic Quinones (in CHCl₃)

Compound no.	λ , nm (log E)
I	251(4.7), 272(4.2), 325(3.5), 310(3.2), 330(3.4)
II	244(4.3), 258(3.2), 270(3.8), 280(3.5), 318(2.4), 388(3.0), 356(3.6), 379(4.0), 400(4.2), 432(4.1), 456(4.2)
III	238(4.4), 242(4.5), 250(4.4), 281(3.7), 290(3.6), 305(3.5), 352(3.8), 365(3.9), 395(3.8), 460(4.0)
IV	235(3.5), 247(3.6), 266(4.6), 278(4.7), 288(4.9), 298(4.0), 330(3.7), 370(2.6)
V	235(4.4), 240(4.3), 246(4.2), 255(4.1), 270(3.1), 302(2.8), 347(2.5), 370(2.6), 438(3.6), 462(3.7)
VI	263(4.1), 282(3.4), 292(3.3), 305(3.2), 354(3.0), 373(3.1), 410(2.6), 418(2.5)
VII	259(4.1), 289(4.0), 301(2.4), 318(2.1), 348(2.2), 370(2.5), 395(3.0), 439(3.8), 464(3.9)
VIII	252(4.0), 287(2.9), 297(2.8), 302(2.5), 346(2.5), 382(2.2), 400(2.3), 430(3.6), 453(3.7), 476(3.5)
IX	239(4.0), 267(3.7), 276(3.8), 328(3.0), 338(3.1), 352(2.9)
X	238(4.5), 252(4.3), 258(4.3), 267(4.1), 286(3.5), 335(2.4), 389(2.7), 409(3.0), 434(4.2), 463(4.4)

chromatographic isolation of the parent PAH's, were employed in the correction of the data obtained from the analysis of reduced PQu's originally present in the PQF's.

Results and Discussion

Chromatographic Isolation and Spectral Analysis of Polycyclic Quinones. Thin-layer chromatographic isolation of three low-molecular-weight quinones (benzoquinone, naphthoquinone, and anthraquinone) has been reported (23) using polyamide layers with various solvent systems. However, the solvent systems used in the above study were unsatisfactory for the resolution of PQu's possessing higher molecular weights.

Several new solvent systems were investigated with the result that a mobile phase of acetic acid:water (19:1, v/v) on Polyamide 11 thin layers (0.25 mm thick) provided the best overall resolution of the PQu's under study. The R_f values for 10 PQu's, using the above chromatographic technique, are summarized in Table II. The PQu's of different molecular weight were readily separated and, in addition, several isomeric compounds were resolved (PQu's of pyrene,

benz[a]anthracene, and benzo[a]pyrene). Acetylated and unacetylated thin-layer plates of polyamide, obtained from commercial sources, were investigated with the result that no discernible difference in the degree of PQu resolution was indicated. It may be possible that the layer becomes progressively acetylated as the acetic acid phase ascends the plate. The amount of water in the mobile phase was also important since acetic anhydride in anhydrous pyridine as a mobile phase failed to move to PQu's from the origin of the plate. The PQu spots on thin-layer plates do not readily fluoresce under long-wavelength ultraviolet light. However, because of the slight background fluorescence on the polyamide plate (19), the PQu's may be detected as quenched spots and, as a result, no destructive spray reagents need be employed for identification. A major disadvantage in the use of polyamide thin-layer plates for quantitative isolation and spectral analysis lies in the fact that nonreproducible recoveries of the PQu's were obtained. Perhaps some type of irreversible adsorption on the layer occurs and, thus, disallows complete recovery of the compounds. However, polyamide thin-layer chromatography may be used for identification purposes when combined with ultraviolet spectral analysis. Another disadvantage arises from the time of development, since approximately 160 min are required for adequate resolution of the individual components in the chromatogram.

Thin layers of magnesium hydroxide have been used for the separation of PAH's (24) and some PQu's (16) and were well suited for both preparative and analytical separations, depending upon the thickness of the adsorbent. The R_f values for the resolution of specific PQu's on thin layers of Mg(OH)₂ (0.25 mm thick), using various chlorinated solvents as the mobile phase, are summarized in Table II. The migration distances and degree of resolution of the PQu's were dependent upon the polarity of the mobile phase. Whereas carbon tetrachloride failed to remove the compounds from the origin of the plate, satisfactory resolution was achieved with both CHCl₃ and CH₂Cl₂, although some overlap between the PQu's of benz[a]anthracene (BaA) and benzo[a]pyrene (BaP) was observed. Although thin layers of Mg(OH)₂ do not provide as good a resolution as thin layers of polyamide for certain isomeric PQu's, the separation of PQu's of different molecular weight may be achieved in a much shorter development time (45 min compared to 160 min for polyamide). Thin-layer chromatography employing adsorbent layers of alumina, silica gel, or acetylated cellulose failed to yield satisfactory resolution of the PQu's.

The positions and intensities of the ultraviolet absorption bands for the synthesized PQu's appear in Table III. Since several of these PQu's are only slightly soluble in solvents, such as benzene or cyclohexane, spectrograde CHCl₃ was used as the solvent for all spectrophotometric measurements. Several PQu's absorb strongly in the visible region while others absorb predominantly in the near-ultraviolet region.

The mass spectral peaks and intensities of PQu's are given in Table IV. Only peaks that correspond to the loss of one or two CO fragments, along with the molecular ion peaks, are reported (i.e., 7,12-BaA-Qu: $m^+ = 258$, $m/e = 230$ for $m^+ - \text{CO}$). Although the isomeric quinones yield the same molecular ion and fragment peaks, the relative intensities of the peaks are quite different and, hence, may be used as an aid in identification.

Application to Air Pollution Analysis. Several attempts have been made to identify polycyclic quinones in polluted air. Falk (25) reported the identification of two oxygenated compounds in air by their absorption and fluorescence spectra after reduction of the compounds to their

Table IV. Mass Spectra of Polycyclic Quinones

Compound no.	M/e (relative intensity)
I	208(100), 180(41), 152(88)
II	232(100), 204(43), 176(99)
III	232(100), 204(66), 176(40)
IV	258(100), 230(37), 202(49)
V	258(28), 230(100), 202(44)
VI	282(100), 254(75), 226(38)
VII	282(100), 254(46), 226(68)
VIII	282(100), 254(25), 226(39)
IX	282(100), 254(48), 226(98)
X	232(100), 304(16), 276(23)

dihydroxy derivatives by distillation with zinc dust. Jäger (10) identified benzo[a]pyrene quinones in polluted air by using fluorescence spectrophotometry at -197°C but could not distinguish which isomer was present. Because of the success in the separation of isomers of specific PQu's in this study, the technique of thin-layer chromatographic isolation in combination with ultraviolet and fluorescence spectrophotometry was used in the analysis of PQu's in particulate extracts obtained from atmospheric sources.

When aliquots of PQF's, obtained from monthly BSF composites, were applied to polyamide thin-layer plates, developed, and viewed under ultraviolet light, the dried chromatograms displayed several spots of which five were tentatively identified as compounds I, VI, VII, VIII, and X. A representative chromatogram of a PQF, resolved into its various PQu's, appears in Figure 2, along with the appropriate internal standards and some PQu's derived from the laboratory photooxidation of BaP and BaA. Specific isolated spots in the PQF, corresponding in fluorescence color and R_f value to standard PQu's, were removed from the chromatogram, eluted, and subjected to ultraviolet absorption spectral analysis. A representative ultraviolet spectrum, displaying the band positions and relative intensities of both internal standard and unknown fraction, is illustrated in Figure 3. Comparison with published spectra (1) indicated that the above spectrum was similar to that of the 1,6-quinone of BaP (compound VII). Compounds I, VI, VIII, and X were similarly identified. Because of the relatively small amounts of PQu's isolated from the PQF's, mass spectra could not be obtained.

Due to the difficulty in the quantitative analysis of such small concentrations by ultraviolet absorption techniques, the quinones were reduced to their parent hydrocarbons and analyzed by fluorescence spectrophotometry. This process increased the sensitivity of the analysis procedure by $10^2\text{--}10^3$ and, as a result, estimation of PQu content could be obtained. The reduced PQF's were subjected to thin-layer chromatography on alumina with hexane:ether (19:1, v/v) as the developing solvent. The dried chromatogram displayed spots due to anthracene ($R_B = 1.15$), benz[a]anthracene ($R_B = 1.09$), benzo[a]pyrene ($R_B = 1.00$) and dibenzo[b,def]chrysene ($R_B = 0.72$). R_B value, when used in thin-layer chromatography, may be defined as the ratio of the distance traveled by a compound to the distance traveled by pure benzo[a]pyrene, acting as an internal standard (20). The fluorescence excitation and emission spectrum of a reduced PQF, that had been separated on alumina (the spot corresponding in fluorescence color and R_B value to standard BaP removed and eluted), is compared to the fluorescence excitation and emission spectrum of a standard

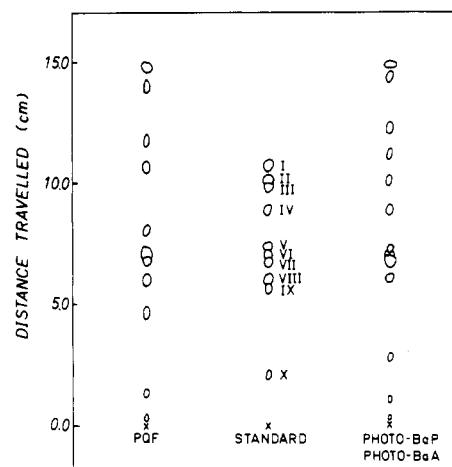


Figure 2. Chromatographic isolation on thin layer of polyamide of PQu's present in ambient particulate matter and photooxidized samples

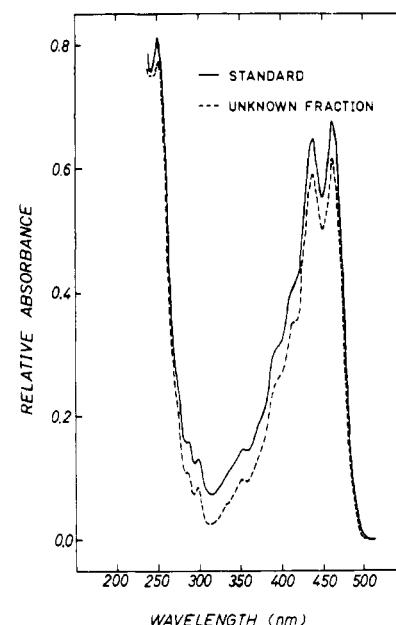


Figure 3. Ultraviolet absorption spectrum of benzo[a]pyrene-1,6-quinone and an unknown constituent of PQF

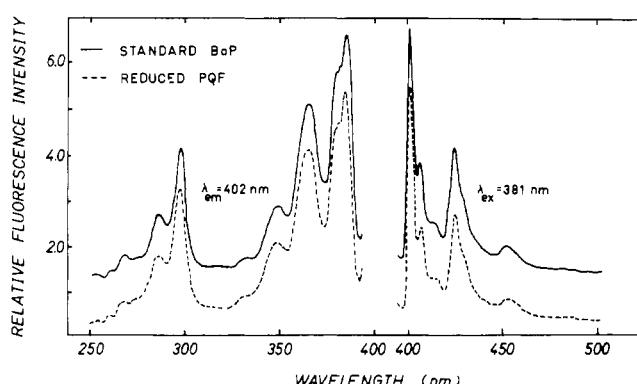


Figure 4. Fluorescence spectrum (uncorrected) of pure benzo[a]pyrene and a reduced constituent of PQF

Table V. Recovery of PAH's Subsequent to Reduction of Polycyclic Quinones

Compound	Recovery, ^{a,b} %	Rel std dev ^b
Anthracene	69.7	10.1
Benz[a]anthracene	84.6	8.7
Benzo[a]pyrene	77.3	10.2
Dibenzo[b,def]chrysene	74.2	9.6

^a Recoveries include reduction, TLC and analysis procedures.

^b Geometric mean of multiple determinations.

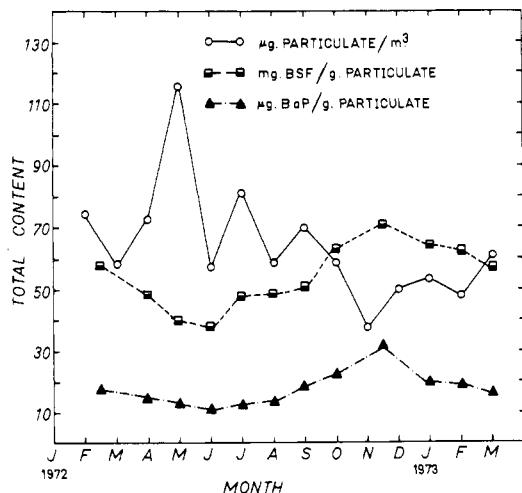


Figure 5. Seasonal variation in the amount of particulate matter, BSF, and BaP

solution of pure BaP in Figure 4. Likewise, the identities of anthracene, benz[a]anthracene, and dibenzo[b,def]chrysene were established. Although the above method does not designate which isomeric quinone was originally present in a BSF, it does indicate which ring structure the PQu possessed prior to reduction. Whereas only qualitative data for specific PQu's could be obtained from the chromatographic isolation on polyamide in conjunction with ultraviolet determinations, both qualitative and quantitative data could be secured by reduction of the PQF, isolation of the products on alumina, and spectral analysis of the parent PAH's by fluorescence techniques. Although the recoveries of the PAH's subsequent to reduction of PQF's were relatively

low, reproducibilities were within analytical limits (Table V).

Levels of suspended particulate matter were highest during the early summer at the York site and gradually decreased to a minimum during early winter (Figure 5). During late winter and early spring, the mass concentration gradually increased. However, the content of BSF and BaP associated with this particulate matter followed essentially the reverse pattern—i.e., minimal values were recorded during summer while maximal values were recorded during winter. Data for BSF, BaP, and other constituents are expressed with respect to weight of particulate matter collected and, thus, it must be assumed that all the compounds are associated with the ambient aerosol and are not in a free state in the atmosphere. This appears to be a valid assumption, as Commins (26) detected no polycyclic organic matter in a free state in ambient samples. Other investigators (27, 28) have speculated that BaP and other arenes appear to be adsorbed primarily on the surface of soot particles.

The amount of PQF found in the collected particulate matter followed a definite seasonal pattern with maximal values occurring in the winter months while the lowest values appeared in the early summer months. The monthly distribution of PQF content was similar to the monthly distribution of BSF content (Table VI), whereas a significantly different trend appeared when the ratio of PQF to BSF was compared on a monthly basis. In this case, the ratio increased to a maximal value during midsummer with lower values being recorded for fall, winter, and early spring sampling periods (Figure 6).

The monthly ambient concentrations of BaP and BaA, along with the concentration of their corresponding quinone derivatives are listed in Table VI. In general, both BaP and BaA content followed similar monthly distribution patterns to the pattern recorded for BSF content—i.e., high content in late spring decreasing to a low in midsummer and then increasing to a maximal value during winter. The concentration of benzo[a]pyrene quinone (BaP-Qu) exhibited the same seasonal distribution as did the seasonal distribution of PQF found in the collected particulate matter. However, the ratio of BaP-Qu to BaP displayed a marked increase during the midsummer months with decreased values for winter and early spring (Figure 6). It is known that most tricyclic or larger hydrocarbons are very readily photooxidized and photooxidation is probably one

Table VI. Seasonal Distribution in Content of Various Fractions Found in Particulate Matter^a

Month	BSF/g particulate, mg	PQF/g particulate, mg	BaP/g particulate, μg	BaA/g particulate, μg	BaP-Qu/g particulate (×10), μg	BaA-Qu/g particulate (×100), μg	BO/g particulate, μg
1972							
Feb.–Mar.	58.2	5.8	17.1	22.2	6.8	2.7	19.2
April	48.4	4.4	14.8	20.7	6.2	5.0	14.4
May	40.1	4.0	12.9	11.6	7.4	4.3	18.1
June	37.7	4.2	10.8	10.1	6.7	7.5	18.0
July	48.0	5.6	12.6	11.3	10.7	9.7	30.5
August	48.5	5.8	13.1	12.6	12.1	11.6	32.1
September	50.6	5.1	18.9	19.2	16.6	17.7	44.6
October	63.2	5.7	22.2	27.2	16.5	22.8	29.3
Nov.–Dec.	70.8	5.7	31.9	35.7	19.5	18.6	18.5
1973							
January	64.5	5.2	19.7	26.2	7.9	2.6	14.2
February	62.3	5.6	19.0	24.3	7.0	2.2	15.4
March	57.2	5.1	16.2	19.3	6.2	1.5	13.6

^a Geometric mean of multiple determinations.

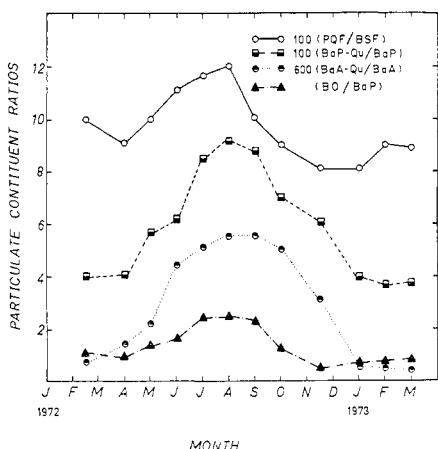


Figure 6. Seasonal trend in the ratio of PQu's to parent hydrocarbons found in suspended particulate matter

of the most important processes in the removal of polycyclic hydrocarbons from the atmosphere. However, the ratio of BaP-Qu to BaP appeared to be significantly low for all sampling intervals. The above observations would indicate either that little BaP is photooxidized to BaP-Qu in the atmosphere or that the quinones are only minor products in the photooxidation of the parent hydrocarbon. Other studies showed that numerous products arise from BaP that has been subjected to ozone and light in a simulated atmosphere while the rate of BaP decomposition is relatively rapid.

The ratio of benz[a]anthracene quinone (BaA-Qu) to BaA displayed the same seasonal distribution as did the ratio of BaP-Qu to BaP (Figure 6, Table VI). However, the ratio of BaA-Qu to BaA appeared to be lower than the ratio of BaP-Qu to BaP by a factor of at least 10. These results would indicate either that BaA is more stable than BaP under photooxidative conditions or that BaA is less stable, but that the end products of the degradation are not quinones but some other products. Equimolar concentrations of pure BaP and BaA were subjected to ozone and radiation in simulated atmosphere (17), and the rates of photooxidation were investigated. More than half the original amount of BaP was degraded after 30 min in the reaction chamber compared to only one-fifth the original amount of BaA. Hence, the conversion of BaP to decomposition products appears to occur at a much faster rate than the conversion of BaA to products. One would expect, therefore, a smaller rate of conversion of BaA to oxygenated products under photooxidation conditions. Some of the oxygenated products, notably the PQu's, from this degradative reaction appear in the chromatogram of Figure 2.

The seasonal trend in the concentration of benzanthrone (BO) in airborne particulate was measured and is reported in Table VI. As in the previous cases, the ratio of BO to BaP increased in midsummer but decreased in late spring, fall, and winter (Figure 6). The concentrations of BO varied by factors of 0.6 to 2.5 times the concentration of BaP for the same sampling period. Other investigators (29) have reported that BO content was highest during the winter and fall quarters, whereas the ratio of BO to BaP was highest during the summer and fall quarters for several sampling locations. Tebbens et al. (27) have proposed that the photooxidation of BaP first yields polycyclic quinones which are further photooxidized to structures similar to 7H-benz(*de*)anthracen-7-one-3,4-dicarboxylic acid. Further photooxidation of this product, or some similar intermediate structure, may then yield benzanthrone. A crude

mixture of the products of BaP recovered from the photooxidation treatment previously described was analyzed for BO content. Fluorescence spectral analysis, subsequent to the thin-layer chromatographic isolation of constituents present in the crude mixture, confirmed the presence of a minute quantity of BO. If relatively similar mechanisms exist for the photooxidation of BaP in a simulated atmosphere as for BaP degradation in the ambient atmosphere, one would expect that the conversion of BaP to BO would occur at a very slow rate. As a result, the photochemical degradation of BaP does not fully explain the relatively high concentrations of BO found in ambient particulate matter.

The difference between summer and winter contents of several arenes in the airborne particulate matter of several locations in Toronto, Ont., have recently been evaluated (20, 21). Of significance is the fact that from a large amount of data, there was a difference in the summer and winter concentrations of arenes in the extract of airborne particulates in most cases. In summer, the amount was 30–70% of that in the winter months at a number of locations. These results may be interpreted as a decrease in arene content by photochemical degradation combined with a difference in fuels and burning methods for the different seasons.

Conclusions

The chromatographic resolution of PQu's derived from PAH's, has aided in the improvement of the analysis of these minor constituents in atmospheric particulate matter. Preliminary separation, using either column or preparative thin-layer chromatography, was required prior to final isolation of PQu's on thin layers of polyamide. Identification of airborne and photosynthesized PQu's was carried out using such instrumental techniques as ultraviolet, visible, and fluorescence spectrophotometry. Because of the trace concentrations of PQu's present in suspended particulate matter, the above techniques were limited to qualitative determinations.

Quantitative data were obtained by reducing the PQu's to their parent aromatic hydrocarbons. Isolation by alumina thin-layer chromatography and quantitative determinations by fluorescence spectrophotometry were then accomplished. Seasonal variations were discovered in the content of BSF, PQF, BaP-Qu, BaA-Qu, and BO, associated with PAH's in suspended particulate matter.

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Avoidance of Lime-Neutralized Iron Hydroxide Solutions by Coho Salmon in the Laboratory

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■ Laboratory avoidance studies indicated that coho salmon, raised in clean water, have shown a definite response to lime-neutralized iron hydroxide suspensions at the range of 4.25–6.45 mg Fe/l. The same species of fish, exposed for several months to different concentrations of the pollutant, has shown almost identical response as the fish raised in the control water.

Throughout the United States, there exist certain sections where the principal contributor to water pollution is acid drainage, most commonly originating from coal-mining activities and characterized by a high dissolved iron content. The passage of water through coal mines where iron pyrites (FeS_2) have been exposed to the oxidizing action of air, water, and bacteria results in the production of acid and the release of soluble ferrous iron (1). When the pH is raised and oxidation takes place (e.g., in a receiving water body), most of the soluble ferrous iron is converted to insoluble ferric species.

The chemical processes in acid mine drainage treatment plants are based on the same principles (neutralization and oxidation) resulting in precipitation of metal compounds which are removed by sedimentation. Under normal treatment plant operation, ambient suspended iron concentrations are greatly reduced, but nevertheless, receiving waters may be furnished with low levels of ferric iron for indefinite periods, while somewhat higher levels of ferric iron enter intermittently when abnormal iron loadings occur.

To provide data useful in the regulation of iron discharges in neutral effluents, this bioassay was undertaken to ascertain the threshold concentration of solution containing suspended ferric hydroxide that would be detected and spontaneously avoided by coho salmon (*Oncorhynchus kisutch*, Walbaum).

Methods and Materials

Physical-Chemical System. The diluent water was pumped from a well (18 m) by a $\frac{1}{3}$ -hp submersible pump to

a 378-l. Nalgene storage reservoir. This holding vessel was equipped with a 1-hp cooling unit, thermostatically controlled to maintain a water temperature of 10.0°C. The most recent diluent water analyses are summarized in Table I.

A modified proportional diluter, based on the design by Mount and Brungs (2) was used to deliver the pollutant. The apparatus simulates the neutralization phase of an acid mine drainage treatment plant. Thus, the basic operation of the dosing equipment entails (a) neutralization of a ferrous sulfate stock solution with lime slurry, and (b) oxidation of the resultant blue-green bivalent iron suspension to the red-brown trivalent iron precipitate, ferric hydroxide. Construction and function of the modified proportional diluter were described by Sykora et al. (3).

From the diluter each concentration of suspended iron was delivered to a 4-l. detention tank connected by a siphon tube to another 4-l. container. For avoidance experiments, the selected iron concentration was siphoned from

Table I. Dilution Water Characteristics

Characteristic	Mean	Std dev
Total iron, mg Fe/l. ^a	0.19	0.18
Total dissolved iron, $\mu\text{g Fe/l.}$	159.0	59.0
Total ferrous iron, $\mu\text{g Fe/l.}$	50.0	81.0
pH	7.76	0.16
Conductance, $\mu\text{mhos/cm}$	3236.0	135.0
Turbidity, JTU	5.3	0.9
Total acidity mg $\text{CaCO}_3/\text{l.}$	2.49	1.31
Total alkalinity, mg $\text{CaCO}_3/\text{l.}$	99.8	2.6
Total hardness EDTA, mg $\text{CaCO}_3/\text{l.}$	159.0	10.6
Hardness Ca^{2+} , mg $\text{CaCO}_3/\text{l.}$	61.0	21.5
Total Ca^{2+} , mg $\text{Ca}^{2+}/\text{l.}$	24.4	8.6
SO_4^{2-} , mg $\text{SO}_4^{2-}/\text{l.}$	43.6	5.9
Total Mg^{2+} , mg $\text{Mg}^{2+}/\text{l.}$	5.66	...
Total Mn^{2+} , mg $\text{Mn}^{2+}/\text{l.}$ ^a	0.03	...
O_2 , % saturation	92.6	3.0
Temperature, °C	10.0	0.6

^a Most of these metals were removed by aeration and settling in the storage tank.

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