ORGANIC SECTION

Spectral and Other Properties of Some Oxygenated Derivatives of Benzo(*a*)pyrene

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The synthesis and spectral properties of some oxidation products of benzo(*a*)pyrene are described. A mixture of 1,6-, 3,6-, and 6,12-diones was separated by thin-layer chromatography on magnesium hydroxide. Vollmann's 3,6-diacetoxybenzo(*a*)pyrene was actually a 1 to 1 mixture of 1,6- and 3,6-isomers, as determined by nuclear magnetic resonance (nmr) spectrometry. Improved methods for preparing 4,5-dione and the corresponding dihydrodiol are described.

Conjugates of some dihydroxybenzo(*a*)pyrenes have been tentatively identified as metabolites of benzo(*a*)pyrene (B[*a*]P) *(4)*. The metabolism of B(*a*)P has been actively studied *(7, 13)*, even in recent years.

Our objective was to prepare 1,6-, 3,6-, 4,5-, and 6,12-diacetoxybenzo(*a*)pyrenes as prototypes of the conjugates and study their ultraviolet and other spectra, so that this information might be used to help identify the metabolites of the hydrocarbon. [Our detailed studies were well under way when Antonello and Carlassare *(1)* published their results.]

Chromic acid oxidation of benzo(*a*)pyrene gives a mixture of three isomeric dione derivatives, which Antonello and Carlassare *(1)* reported separating by column chromatography on aluminum oxide. We *(8)* have found that chromatography on thin layers of magnesium hydroxide is a convenient and economical alternative method for resolving this mixture. Preparative plates of this adsorbent were each streaked with 3–4 mg of the oxidation mixture and developed in chloroform. There were three distinct bands in the resulting chromatogram. Elution of these yielded crystalline compounds agreeing in melting point and ultraviolet spectrum with the 6,12-, 1,6-, and 3,6-diones (in order of decreasing R_f). These were recrystallized for use in the synthetic steps.

There appears to be a discrepancy in the literature regarding the melting point of 3,6-diacetoxybenzo(*a*)pyrene. Vollmann and collaborators *(14)* have described this compound as a solid melting sharply at 204°C, while Antonello and Carlassare *(1)* reported 227° as the melting point of their product. Moriconi et al. *(9)* also attempted to explain this discrepancy by suggesting that Vollmann's 3,6-diacetate was a mixture of 3,6- and 4,5-diacetates.

In an effort to resolve this discrepancy, we subjected the chromatographically pure 3,6-dione isolated above to reductive acetylation. The nmr spectrum of the product, mp 227°C, revealed the presence of two different acetoxy groups (singlets of equal intensity at 7.29 and 7.41τ). This observation, together with the known stereochemical purity of the starting 3,6-dione, confirmed the identity of this compound as pure 3,6-diacetoxybenzo(*a*)pyrene.

Repetition of Vollmann's procedure, on the other hand, yielded a product (mp 203–04°C) having an nmr spectrum consisting in the alkyl region of three singlets at 7.29, 7.41, and 7.44 τ (relative intensities of 2:1:1). Since pure 1,6-diacetoxybenzo(*a*)pyrene shows singlets of equal intensity at 7.30 and 7.45 τ in the solvent used above (dimethyl sulfoxide-d_6 with added tetramethylsilane), we have concluded that the analytically pure solid with a sharp melting point of 203–04°C of Vollmann et al. is actually a 1 to 1 complex of the 1,6- and 3,6-isomers. This interpretation is consistent with the material's origin —i.e., from crystallization of the crude product of the reductive acetylation of the mixed diones obtained by chromic acid oxidation of benzo(*a*)pyrene.

Attempts to separate this mixture by chromatography on acetylated cellulose have failed, but eluates from alumina columns appeared from their nmr spectra to be enriched in the 1,6-isomer. It was not clear whether this enrichment was due to chromatographic resolution of the complex or to the selective destruction of the 3,6-derivative (an orange-red dione developed on the alumina column).

Cook and Schoental *(3)* described the addition of OsO_4 to B(*a*)P. KOH-mannitol decomposition of the osmate adduct, carried out according to these authors, yielded dihydrodiol, the infrared spectra of which contained carbonyl bands at 1750, 1660, and 1610 cm^{-1}. Cook and Schoental *(3)* described the formation of quinones during their preparations (see Table VII for infrared bands of diones at 1660 and 1610 cm^{-1}). The band at 1750 cm^{-1} could be due to α-methylene ketone *(6, 11)*.

By using an improved method in which Na_2SO_3 and $KHCO_3$ were used in place of KOH-mannitol, as suggested by Bernstein and Littell *(2)*, practically colorless dihydrodiol, containing some carbonyl compounds as impurities, was obtained.

Cook and Schoental *(3)* used a mineral acid–catalyzed chromic acid oxidation of the dihydrodiol to prepare the 4,5-dione. In our work the acid-sensitive dihydrodiol was oxidized to the dione in high yield with CrO_3 in pyridine *(10)*. The dione thus produced was converted to the diacetoxy derivative in the usual manner *(1, 9, 14)*.

For the sake of convenience and ease of reference, the melting points, ultraviolet, infrared, and mass spectra of the diones and the diacetates and the nmr spectra of the diacetates from our preparations are presented in Tables I to X. For the routine identification of metabolites *(11–13)* the ultraviolet spectra were useful. If metabolites can be pooled, the other spectral identification can also be used.

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Table I. Melting Points of Benzo(*a*)pyrenediones

Compound	Mp, °C
1. 1,6-	291 (chlorobenzene)
2. 3,6-	292–93 (AcOH)
3. 4,5-	258–60 (three crystallizations from xylene)
4. 6,12-	327 (dichloromethane)

Table II. Melting Points of Diacetoxybenzo(*a*)pyrenes

Compound	Mp, °C
1. 1,6-	245
2. 3,6-	227
3. 4,5-	258–60
4. 6,12-	250

Mps of 1,6-, 3,6-, 6,12-, and 4,5-diacetoxybenzo(*a*)pyrenes are of samples prepared by us. Mixture melting point of 4,5-dione and diacetoxy derivative gave depression.

Table III. Ultraviolet Spectra of Benzo(a)pyrenequinones: *nm*(ϵ)

1,6-Quinone	3,6-Quinone	4,5-Quinone	6,12-Quinone
		267 (5198)	282 (2352)
		275 (5876)	291 (2462)
315 (196)			303 (2106)
327 (190)	290 (912)		352 (1012)
	303 (639)	312 (859)	370 (1163)
	317 (693)		
	347 (617)		
	385 (365)	337 (1197)	
	405 (333)	350 (927)	

Chloroform used as solvent.

Table IV. Ultraviolet Spectra of Diacetoxybenzo(*a*)pyrenes

1,6-Diacetoxy	3,6-Diacetoxy	4,5-Diacetoxy	6,12-Diacetoxy
256 (40,888)	256 (32,016)	256 (43,792)	253 (43,792)
262 (47,703)			
272 (28,395)	266 (34,960)	266 (52,992)	266 (50,784)
284 (41,797)	278 (18,400)	275 (29,072)	274 (32,384)
297 (52,246)	289 (34,592)	287 (48,576)	286 (47,840)
337 (5,224)	302 (46,736)	298 (61,824)	297 (59,984)
355 (13,402)	337 (4,048)	333 (5,888)	337 (6,256)
	355 (9,568)	349 (13,248)	355 (14,720)
373 (27,032)		367 (25,392)	373 (28,336)
		381 (19,136)	387 (23,184)
382 (20,217)			
	373 (18,400)	387 (29,440)	393 (31,648)
		394 (11,040)	
388 (23,624)	383 (13,984)	403 (1,472)	407 (7,728)
393 (30,439)	387 (15,824)		
	394 (19,872)		

Figures in parentheses are ϵ values of peaks. Alcohol (95%) used as solvent.

Table V. Ultraviolet Absorbance-Concentration Relationship of Benzo (*a*) pyrenediones

Compound	μg/ml (nm)
1. 1,6-	1532 (330)
2. 3,6-	772 (384)
3. 4,5-	227 (337)
4. 6,12-	242 (370)

Weighed amounts of diones were dissolved in chloroform and ultraviolet spectra determined between 410 and 250 nm. Concentration of a particular dione, at respective wavelengths mentioned above, giving absorbance 1, was then calculated. Since we used the values for determining the concentration in metabolic samples *(11–13)*, longer wavelengths were selected. In metabolic samples there is interference at lower wavelengths.

Experimental

The melting points are uncorrected. Infrared spectra were obtained with a Beckman IR-18 spectrophotometer, nmr spectra with a Varian HA-100 spectrometer in DMSO-d_6 with $Si(CH_3)_4$ as internal standard, mass spectra with an AEI MS-9 operating at an ionizing potential of a 70-eV instrument, and ultraviolet spectra with a Cary 15 spectrophotometer. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Oxidation of B(a)P to dione. A literature method was used for obtaining the dione mixture *(14)*.

TLC separation of dione mixture. Preparative tlc on magnesium hydroxide plates was performed essentially as previously described *(8)*. The developer was reagent grade chloroform. Adsorbent layers were 1 mm thick.

OsO_4 adduct of B(a)P. The following operations were carried out under an atmosphere of Ar or N_2 gas. The solid OsO_4 adduct from 2 grams of B(*a*)P was washed with 2 × 20 ml of benzene and 2 × 10 ml of ether to obtain a brown solid which was dried to a constant weight (4 grams) in a vacuum desiccator. The brown osmate had λ $CHCl_3$ (ϵ) 267 (32,108), 274 (35,430), 298 (13,563), 314 (9,964), 327 (8,027), 350 (4,152), 367 (3,598), 379 (3,044), 387 (3,321), 403 (2,214). Assuming two molecules of pyridine in the complex *(5)*, the molecular weight was taken as 664.4. The mass spectrum had ions at *m*/*e* 252, 250, and 224. Its infrared spectrum taken in mineral oil did not show any carbonyl bands. After ultraviolet and mass spectra, the osmate was added, all in one portion with stirring, to a solution of 72 grams of Na_2SO_3 and 72 grams of $KHCO_3$ in 450 ml of water taken in a three-necked flask. After 30 minutes' stirring, 225 ml of methanol was added and stirring was continued for 3 more hours. The brownish mixture was filtered through a filter paper arranged under N_2. The brownish solid on the funnel was washed while stirring with 100 ml of water, 100 ml of methylene chloride, and 200 ml of hot tetrahydrofuran. The combined liquid portions were extracted with 4 × 300 ml of methylene chloride. The lower methylene chloride layer was washed with an aqueous solution (45 ml) of 7.2 grams of $KHCO_3$ and 7.2 grams of Na_2SO_3 and then with 2 × 20 ml of water. The CH_2Cl_2 extract dried over anhydrous Na_2SO_4 was distilled under reduced pressure to obtain 2.0 grams of a brown solid. Three crystallizations from 80% alcohol (Darco G-60, 0.5 gram each) followed by one crystallization from CH_2Cl_2 yielded 1.3 grams of an off-white solid, mp 210–12°C with previous softening at 206°C.

Anal. Calculated for $C_{20}H_{14}O_2$: C 83.90, H 4.93. Found: C 83.28, H 4.81; C 83.33, H 5.10 λ 95% alcohol (ϵ) 263 (35,615), 272 (54,475), 297 (10,894), 310 (10,475), 323 (9,427); ν Nujol cm^{-1} 3400, 3270 (bonded OH), 1300 (OH bending), 1012, 1035, and 1075 (C—OH stretch). In some experiments, products contained traces

Table VI. Ultraviolet Absorbance-Concentration Relationship of Diacetoxybenzo(*a*)pyrenes

Compound	μg/ml (nm)
1. 1,6-	12.1 (393)
2. 3,6-	18.5 (394)
3. 4,5-	12.3 (387)
4. 6,12-	13.2 (393)

Spectra taken in 95% alcohol between 410 and 250 nm. Concentration, at respective wavelengths mentioned above, for which absorbance was 1, was then calculated.

Table VII. Infrared Carbonyl Bands of Benzo(*a*)pyrenediones

Compound	Ir peaks, cm^{-1}
1. 1,6-	1640 (s) (CH_2Cl_2)
2. 3,6-	1642 (s) (mull)
3. 4,5-	1660, 1610 (s) (mull)
4. 6,12-	1650, 1635 (CH_2Cl_2)

Table VIII. Infrared Spectra of Diacetoxybenzo(*a*)pyrenes

Compound	Ir peaks, cm^{-1}
1. 1,6-	1760
2. 3,6-	1762
3. 4,5-	1770
4. 6,12-	1760

Spectra taken in CH_2Cl_2.

of carbonyl compounds (1730, 1660, 1610 cm^{-1}). The dihydrodiol was practically insoluble in CCl_4, CS_2, and CH_2Cl_2. The mass spectrum had ions at 286.0991 ($C_{20}H_{14}O_2$), 269.0968 ($C_{20}H_{13}O$), 268.0991 ($C_{20}H_{12}O$), 252.0935 ($C_{20}H_{12}$), and 239.0867 ($C_{19}H_{11}$).

The 4,5-dione was prepared in the following manner. Two and four-tenths grams of CrO_3 were added, in portions of 0.5 gram, to 24 ml of anhydrous pyridine cooled externally with ice. After 15-minutes standing this mixture was added to an ice-cooled pyridine (24 ml) solution of 2 grams of freshly prepared B(*a*)P dihydrodiol.

After overnight stirring, 60 ml of water was added; the orange solid that separated was filtered off and washed with 3 × 20 ml of water; the combined aqueous washings were extracted with 3 × 100 ml of benzene. The benzene extract was washed and dried and the solvent removed. The residue was combined with the main portion of the air-dried 4,5-dione. Yield (crude): 1.7 grams. The solid was crystallized three times from xylene to obtain bright orange crystals, mp 258-60°C *(9)*.

Table IX. Mass Spectra of Benzo(*a*)pyrenediones

Compound	Spectra
1. 1,6-	282 (100), 254 (48), 226 (94)
2. 3,6-	282 (100), 254 (26), 226 (69)
3. 4,5-	282 (22), 254 (100), 226 (44)
4. 6,12-	282 (53), 254 (100), 226 (66)

Figures in parentheses are relative intensities of peaks.

Table X. Mass Spectra of Diacetoxybenzo(*a*)pyrenes

Compound	Spectra
1. 1,6-	368 (5), 326 (10), 284 (100), 254 (13), 226 (68)
2. 3,6-	368 (13), 326 (24), 284 (100), 254 (11), 226 (41)
3. 4,5-	368 (9), 326 (11), 284 (84), 254 (95), 226 (100)
4. 6,12-	368 (15), 326 (33), 284 (100), 254 (16), 226 (34)

Figures in parentheses are relative intensities of peaks.

Anal. Calculated for $C_{20}H_{10}O_2$: C 85.09, H 3.57. Found: C 85.10, H 3.59. ν Nujol 1660 and 1610 cm^{-1}. $\lambda CHCl_3$ (ϵ) 267 (5,198), 275 (5,876), 312 (859), 337 (1,197), 350 (927). Its mass spectrum had ions at *m/e* 282, 254, and 226 (see Table IX).

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