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PHOTOCHEMICAL COUPLING OF BENZO[a]PYRENE
WITH 1-METHYLCYTOSINE, PHOTO-ENHANCEMENT
OF CARCINOGENICITY

E. Cavalieri and Melvin Calvin

January 1971

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PHOTOCHEMICAL COUPLING OF BENZO[a]PYRENE WITH 1-METHYLCYTOSINE;
PHOTO-ENHANCEMENT OF CARCINOGENICITY

E. Cavalieri* and Melvin Calvin

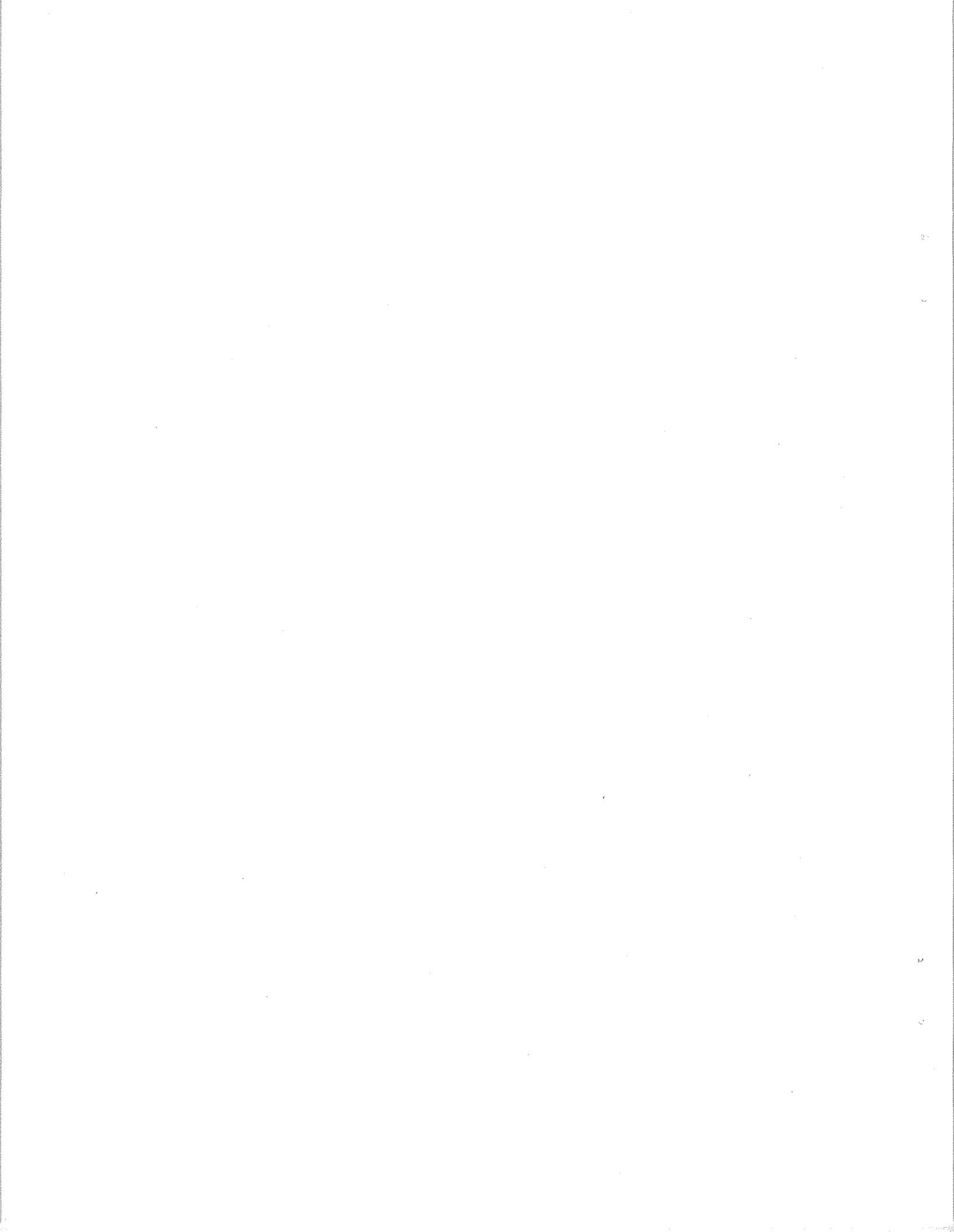
Laboratory of Chemical Biodynamics[†], Lawrence Radiation Laboratory,
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Running Title:

Photochemical Coupling of Benzo[a]pyrene with Methylcytosine

*Damon Runyon Fellow, 1968-1970

[†]This work was supported, in part, by the U.S. Atomic Energy Commission.



Abstract--Irradiation of the carcinogenic benzo[a]pyrene (I) with 1-methylcytosine hydrochloride IIa (molar ratio 1:10) at 3500 Å in methanol-acetone produces the 6-(1-methylcytos-5-yl)-benzo[a]pyrene (III). The structure of the product shows the hydrocarbon bound through the most active 6-carbon atom to the nucleophilic 5-position of the base. In the second part, the possibility of observing carcinogenic effects on the mouse skin from non-carcinogenic hydrocarbons under the action of UV light is investigated. Both experiments provide evidence that the 4,5-double bond (K region) of I presumably does not play a role in triggering the cancer process.

INTRODUCTION

The Pullmans' theoretical attempt aiming to correlate the molecular reactivity of aromatic hydrocarbons to their carcinogenicity has been popular for some time. Pullman and Pullman [1,2] have associated the carcinogenic activity of these compounds with the presence of a chemically reactive phenanthrene type double bond (K region). They have suggested further that the primary process in cancer induction is an addition reaction of the cellular component at the K region. In order to determine whether or not such theoretical assumptions were correct, we have undertaken two rather different experiments. The first one has been a purely photochemical excitation of the carcinogenic benzo[a]pyrene in the presence of a nucleic acid component, methylcytosine. Under these

conditions we might expect a cyclobutane derivative involving the 4,5-double bond of the hydrocarbon (K region) and the 5,6-double bond of the base. However, instead of the cyclobutane adduct, the photoproduct was identified as the 6-(1-methylcytos-5-yl)-benzo[a]pyrene (III). The first part of the paper deals with the elucidation of the photoproduct structure in connection with previous data in the literature [3,4] suggesting cyclobutane structures for similar photochemical products.

In the second part, a photobiological experiment using mice is described. It has been demonstrated that cancer induction on the mouse skin is increased by UV light [5] when a defined concentration of benzo[a]pyrene and intensity of radiation are utilized. The photodynamic action of the aromatic hydrocarbon might be interpreted in terms of excitation of the aromatics with resulting increased binding to the cellular receptor sites. With this idea in mind, the possibility of observing carcinogenic effects from normally non-carcinogenic hydrocarbons, under the action of the UV light, was investigated.

MATERIALS AND METHODS

Materials

Irradiations were carried out in a quartz vessel, using a Rayonet Photochemical Reactor, Model RPR-208 (8 low pressure Hg lamps, 3500 Å). The alumina used for column chromatography was obtained from Woelm and and activity described is in accordance with the prescriptions of the Company. Thin-layer chromatography was carried out on Eastman Chromagram sheets coated with silica gel containing a fluorescent indicator. Preparative thin-layer chromatography was done on glass plates coated with

1 mm thick silica gel containing fluorescent indicator. Melting points were determined on a Büchi apparatus and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer, Model 257. The NMR spectra were scanned on a Varian high resolution HR 220 MHz spectrometer with tetramethylsilane as internal standard.

The UV spectra were recorded on a Cary Model 14 recording spectrophotometer. The 100 watt UV lamp for irradiating mice was a Black-Ray, Model B-100A, with flood bulb (maximum output at 3660 Å) and 5-inch round filter produced by Ultraviolet Products, Inc. (San Gabriel, Calif.).

The Swiss albino mice were obtained from Bio-Sciences Laboratory (Oakland, Calif.).

Experimental details

1-Methylcytosine (II). 1-Methylcytosine was obtained from Cyclo Chemical and had m.p. 305-307° (lit., m.p. 303° [6]). The NMR (in dimethyl-d₆-sulfoxide) (Figure 5A) had absorptions at δ 3.15 (3H, singlet), 5.54 and 7.44 (1H each, doublet, J = 7.0 Hz) and 6.92 (2H, broad singlet).

1-Methylcytosine hydrochloride (IIa). 1-Methylcytosine (1.950 g, 1.56 × 10⁻² moles) was dissolved in 150 ml of methanol. To that solution was added an excess of hydrochloric acid dissolved in methanol. Upon dilution with 300 ml of ether the 1-methylcytosine hydrochloride (2.260 g, 90%) precipitated; its m.p. was 292-293°.

Photolysis of benzo[a]pyrene (I) and 1-methylcytosine hydrochloride (IIa). A solution of I (0.400 g, 1.59 × 10⁻³ moles) in 120 ml of distilled acetone was mixed with a solution of 1-methylcytosine hydrochloride (2.570 g, 1.59 × 10⁻² moles) in 280 ml of absolute methanol. The resulting solution was bubbled with a stream of oxygen-free nitrogen for 1 hr and then irradiated at 3500 Å for 24 hr. The tlc on silica gel using acetone-

benzene-water (70:20:10) indicated a small amount of a new compound (corresponding approximately to 10% of I), showing the *Rf* between the two starting materials, which were still present in large amount. After evaporation of the solvent, the residue was taken up in ether and most of I passed into solution. The insoluble material was dissolved in a mixture of water-chloroform. After extraction the organic layer which contained the new product and some residual I was washed with 5% sodium carbonate and further washed with an aqueous saturated ammonium sulfate solution. The chloroform solution was dried (Na_2SO_4) and, after evaporation, was chromatographed on Woelm alumina activity IV. The first fractions, eluted with benzene-chloroform (1:1) contained 6-(1-methylcytos-5-yl)-benzo[a]pyrene (III) (30 mg). The new compound was further purified by preparative tlc on silica gel and recrystallized from acetone-hexane. A yellow solid with m.p. 331-333° (dec.) was obtained.

Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_3\text{O}$: C, 79.56; H, 5.07; N, 11.13

Found: C, 79.46; H, 5.22, N, 10.61

The infrared spectrum (Figure 2) showed bands at 3520, 3460, 3400, 1655, 1590 cm^{-1} . The UV spectrum (Figure 3C) had maximum absorptions at 258, 268, 278, 290, 302, 340, 357, 375, 395, 407.5 nm. The NMR spectrum (Figures 5B and 6A) possessed absorptions at δ 3.51 (3H, methyl group, singlet), 4.52 (1H, NH broad band exchanged with a drop of D_2O , added to the chloroformic solution of III), 6.64 (1H, NH broad band exchanged with D_2O), 7.35 (1H, $\text{H}_{6\text{MC}}^*$, singlet), 7.70 - 7.89 (3H, H_2 , H_8 , and H_9 , multiplet), 7.92 (1H, H_4 , doublet, $J = 8.8 \text{ Hz}$), 7.99 (1H, H_1 , doublet, $J = 7.5 \text{ Hz}$), 8.08 (2H, H_3 and H_5 , doublet), 8.24 (1H, H_7 , doublet, $J = 7.0 \text{ Hz}$), 8.34 (1H, H_{12} , doublet, $J = 8.8 \text{ Hz}$), 9.02 (1H, H_{11} , doublet, $J = 8.8 \text{ Hz}$, and 9.04 (1H, H_{10} , doublet).

*Proton at the 6-position of the methylcytosine moiety.

1-Methyl-5,6-dihydrocytosine. 1-Methyl-5,6-dihydrocytosine was prepared by hydrogenation of 1-methylcytosine according to the method of Green and Cohen [7] with some modifications. 1-Methylcytosine (125 mg, 1×10^{-3} moles) was dissolved in 40 ml of absolute methanol. To that solution 5% of rhodium on alumina (200 mg) was added. When one equivalent of hydrogen was consumed, the reaction was terminated. After evaporation of the solvent, the solid obtained revealed by tlc (acetone-benzene-water, 70:20:10) the new product with traces of 1-methylcytosine. Two recrystallizations from methanol-acetone removed the starting material. The purified product had m.p. 223-225° (lit., m.p. 223-25° [8]). The NMR spectrum (in dimethyl-d₆-sulfoxide) showed at δ 2.79 (3H, singlet), 2.41 and 3.20 (each 2H, triplet, $J = 7.0$ Hz) and 7.70 (2H, broad singlet). The NMR spectrum in chloroform-d, in spite of the very poor solubility of the compound, exhibited at δ 2.93 (3H, singlet), 2.65 and 3.33 (each 2H, triplet, $J = 7.0$ Hz).

RESULTS

Photochemical results

Photolysis of benzo[a]pyrene (I) and of 1-methylcytosine hydrochloride (IIa). The mixture of benzo[a]pyrene (I) (0.400 g) and 1-methylcytosine hydrochloride* (IIa) (2.570 g) (molar ratio 1:10) when irradiated in 400 ml of a solution of methanol acetone[†] (70:30) at 3500 Å using a Rayonet Photochemical Reactor for 24 hr, seemed to react according to the scheme in Figure 1.

*The protonation of the nitrogen at the 3-position has been clearly demonstrated by Miles *et al.* [9].

[†]In an alternate run, water replaced methanol with the same results.

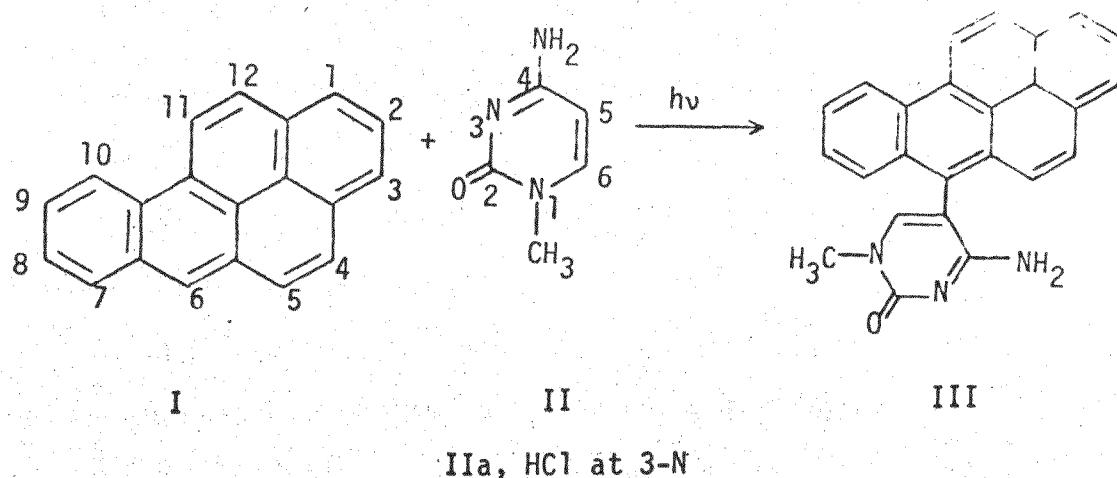


Figure 1

Aprotic solvent systems, such as benzene-acetone (70:30) or pure acetone, does not move compound III on silica gel tlc. However, a solution containing only 2% of water, e.g., acetone-benzene-water (58:40:2), is sufficient to displace the compound. No decomposition of the III is indicated by tlc and UV spectra after the compound is boiled for 24 hr in methanol-water. The product is equally stable after treatment with 1 N hydrochloric acid for 2 hr at 100° or with 1 N sodium hydroxide for 12 hr at room temperature.

When a mixture of I and II (rather than IIa) was irradiated, only trace amounts of the new product were revealed by tlc. The comparison of this product with the compound III showed the same Rf on tlc and the same UV spectrum. In contrast, the non-carcinogenic benzo[e]pyrene VII and 1-methylcytosine hydrochloride IIa or 1-methylcytosine II, when photolyzed under the same conditions, formed no products visible on tlc or, if any, less than 1%.

An attempt to get the coupling chemically between the compounds I and IIa (molar ratio 1:10) in acetone-water (66:33), using as promoters

either iodine or hydrogen peroxide in the presence of ferrous sulfate, was unsuccessful. This experiment was suggested since a covalent binding between I and DNA [10] has been induced by iodine, or hydrogen peroxide (in the presence or absence of ferrous ion), or the ascorbic acid model hydroxylating system.

Elucidation of the photoproduct (structure III). The structure of compound III has been elucidated by the elemental analysis (see Experimental), and the infrared, ultraviolet, and nuclear magnetic resonance (NMR) spectra.

The infrared spectrum (Figure 2) shows characteristic absorptions at 3520 and 3400 cm^{-1} attributable to the NH primary amine, 1655 and 1590 cm^{-1} , ascribable respectively to the carbonyl vibration of a δ -lactam and to the bending vibration of an amine. The disappearance of the characteristic strong absorption of the benzo[a]pyrene at 870 cm^{-1} between the absorptions at 840 cm^{-1} and 905 cm^{-1} (see Figure 2), corresponding to the out-of-plane bending band of the CH group at the 6-position (penta-substituted benzene [11]) offers some suggestion that such a position is involved in the attachment to the base.

The UV spectrum (Figure 3C) has similar absorption maxima to benzo[a]-pyrene (Figure 3A) with all the bands shifted to longer wavelengths by 3-10 $\text{m}\mu$. This displacement toward the red is the same as is found in 6-methylbenzo[a]pyrene (Figure 3B). The molar absorption coefficients for the three compounds, corresponding to the wavelengths of maximum absorption are reported in Table I. The striking similarity of the UV spectra of the methyl- and of the methylcytosyl-benzo[a]pyrene strongly suggests for the latter the attachment to the hydrocarbon through a substituting position.

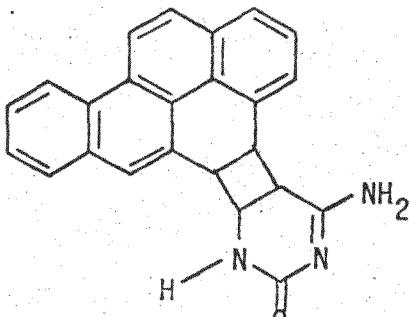
Before discussing the NMR spectrum of the photoproduct III, which will enable us to establish the specific points of linkage of the base

TABLE I: Molar Absorption Coefficients [$\lambda_{\max}(\epsilon_{\max})$].

Benzo[a]pyrene	254.5(53,100) 265(56,500) 272(36,400)
	284(49,000) 296(59,500) 332(4930)
	347(11,900) 365(22,900) 385(24,900)
	404(3150)
6-Methylbenzo[a]pyrene	256(31,000) 267(34,200) 277(18,200)
	288(32,400) 300(45,800) 340(4550)
	356(11,500) 374(21,600) 394(22,800)
	409(5000)
6-(1-Methylcytos-5-yl- benzo[a]pyrene	258(62,700) 268(64,000) 278(43,000)
	290(50,300) 302(48,700) 340(6080)
	357(13,600) 375(27,800) 395(30,600)
	407.5(9430)

and of the hydrocarbon, it is useful to consider the structures previously proposed for compounds of this type.

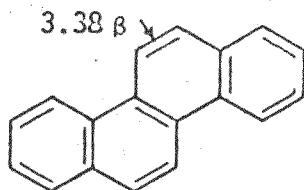
Rice [3] obtained stable products in small amounts when benzo[a]pyrene was irradiated in the presence of uracil, thymine, cytosine, 5-methylcytosine, guanine and 6-azathymine. The UV spectra of these compounds showed the same absorption maxima and were similar to that in Figure 3C. He proposed for cytosine and benzo[a]pyrene the cyclobutane adduct IV, in which the 4,5-double



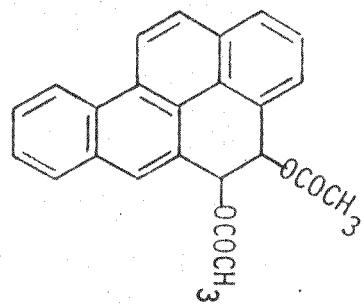
IV

bond of the hydrocarbon is fused with the 5,6-double bond of the base. The 4,5-double bond of the benzo[a]pyrene is the K region proposed by the Pullmans (see Introduction). The rationale of the Pullmans' theory contributed largely to the formulation of the adduct IV. This structure offers two major discrepancies. (i) The UV spectrum of the hydrocarbon, in which the 4,5-double bond is reduced as in IV may be supposed to be similar to the UV spectrum of chrysene, but in fact, it was not (see Figure 2C). The comparison of the UV spectra of chrysene (V) and 4,5-diacetoxy-4,5-dihydro-benzo[a]pyrene (VI),* as shown in Figure 4, are truly similar; therefore, the structure IV for the photoproduct can be regarded as unlikely. (ii) The same UV spectra were obtained from the photoproducts of benzo[a]pyrene and pyrimidine bases or purine bases as, e.g., guanine. While it is easy to

*This compound was prepared in the manner described by Cook and Schoental [12].



V



VI

speculate about the possibility of a cycloadduct of the hydrocarbon with the active 5,6-double bond of the pyrimidine bases, it is a bit more far fetched to expect the same derivative with guanine.

The latter argument is also valid in causing serious doubts about the structure of the cyclobutane adducts proposed by Antonello *et al.* [4] for the same photochemical products. In this case the 7,8-double bond of the hydrocarbon is suggested to form cycloaddition with the nucleic acid bases. The structure of these photoproducts was suggested since there does exist a similarity between the UV spectra of these compounds and the UV spectrum of 7,8-dihydrobenzo[a]pyrene.

The analysis of the 220 MHz NMR (Figure 5B) shows the characteristic methyl protons of the cytosine moiety at δ 3.51. The same protons in the NMR spectrum of 1-methylcytosine in dimethyl-d₆-sulfoxide are at δ 3.15. The two broad singlets at δ 4.52 and 6.64 are exchangeable when a drop of deuterated water is added, and thus correspond to the amino protons. In 1-methylcytosine these two acidic protons possess a unique chemical shift at δ 6.92. The difference of their chemical shifts in the compound III results presumably from hindered rotation of the amino group, as already suggested earlier for the cytosine hydrochloride [13], and for the 1-methylcytosine hydrochloride [9].

The two doublets at δ 5.54 and 7.44 in 1-methylcytosine (Figure 5A), corresponding respectively to the protons in the 5- and 6-positions, have disappeared in compound III (Figure 5B). This might imply, at first glance, that the 5,6-double bond of II is involved in the reaction with the hydrocarbon. However, if this bond has been reduced, the corresponding protons must be strongly shifted to the higher field, as can be observed from the NMR spectrum of 5,6-dihydro-1-methylcytosine in chloroform-*d* (see Experimental), where the protons in the 5- and 6-positions are found at δ 2.65 and 3.33, respectively.

The expanded spectrum of III in the low field region (Figure 6A) permits one to visualize the aromatic protons. The integrated spectrum shows twelve protons determined relative to the protons of the methyl group (Figure 5B) and provides the relative ratio from left to right as 2:1:1:2:1:1:3:1. Comparison of this spectrum with the spectra of benzo[a]pyrene (Figure 6B) and 6-methylbenzo[a]pyrene [14], which have been interpreted, shows the absence of the characteristic singlet peak at δ 8.41, corresponding to the proton in the 6-position. A similar absence has been observed in the spectrum of the 6-methylbenzo[a]pyrene. Therefore, the hydrocarbon is substituted at the 6-carbon atom. The two angular protons H_{10} and H_{11} are clearly identified by their largest down-field shift. The H_{12} is also assigned on the basis of the same coupling constant as H_{11} and about the same chemical shift (slightly deshielded) as in benzo[a]pyrene. The two broad bands centered at δ 8.68 and 9.36 are spinning side-bands.

The other protons of the hydrocarbon moiety are only tentatively assigned. The doublet at δ 8.24 ($J = 7.0$ Hz) is probably the proton in the 7-position, which is not deshielded compared to the benzo[a]pyrene,

although the presence of the base substituted in 6-position should provide a deshielding effect: The H_4 is suggested to be the doublet at δ 7.92 from its chemical shift and coupling constant ($J = 8.8 \text{ Hz}$). The chemical shift and the coupling constant ($J = 7.5 \text{ Hz}$) also suggest the identity of H_1 . In the higher field the complex multiplet centered at δ 7.80 contains three protons, which from their chemical shift are tentatively assigned as the protons at the 2-, 8-, and 9-positions. The two protons at δ 8.08 are the H_3 and H_5 . This band system is a superimposition of two doublets. The singlet at δ 7.35, that was not found in the spectra of benzo[a]pyrene and 6-methylbenzo[a]pyrene must necessarily belong to the methylcytosine moiety. The chemical shift is consistent with the proton at the 6-position. The methylcytosine in dimethyl-d₆-sulfoxide (the base is insoluble in chloroform) shows this signal at δ 7.44. The slight shift difference is compatible with the solvent effect. The spectra of 5,6-dihydro-1-methyl-cytosine in chloroform-d and dimethyl-d₆-sulfoxide present a shift difference of the respective protons of the same order of magnitude (see Experimental). The absence of the signal corresponding to the proton in the 5-position and the resulting singlet in the 6-position clearly signify that the base is substituted at the 5-position.

The UV spectrum, previously discussed, has the same absorption maxima as the 6-methylbenzo[a]pyrene, indicating that the planar ring of the pyrimidine base is not conjugated to the aromatic hydrocarbon and thus, must be perpendicular to it.

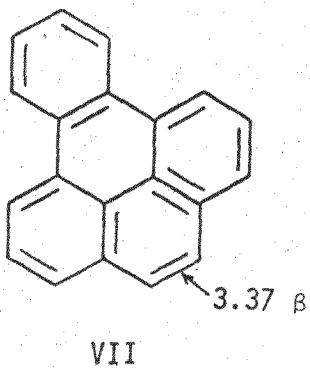
Photobiological experiments and results

Negative photo-carcinogenic effect of non-carcinogenic hydrocarbons.

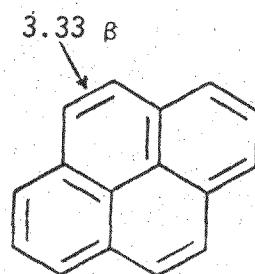
The capacity of binding with the cellular component, in accordance with the Pullman theory (see Introduction) is presumed to be directly related

to the presence of the electronically rich K region. If the energy required to localize the two electrons is below a set value* the aromatic hydrocarbon may form an addition reaction with a biological receptor and thereby induce cancer.

The non-carcinogenic hydrocarbons benzo[e]pyrene (VII) and pyrene (VIII) were chosen, since they are characterized by a similar structure to the benzo[a]pyrene (I) (K value, 3.23β) and possess K values only slightly



VII



VIII

higher than the limit of carcinogenicity.* The latter reason was likewise responsible for the choice of chrysene (V).

The experiment was carried out on 180 mice. Swiss albino mice of both sexes, seven weeks old, were used. The mice were shaved once a week in the interscapular area and painted twice a week with 0.1 mg of benzo[a]pyrene (I) in acetone (one drop of acetone solution delivered by an automatic dispenser) or with the corresponding molar amount of the other non-carcinogenic hydrocarbon (vide supra). They were fed with Simonsen food and water was provided ad libitum. The 180 mice were divided into nine groups treated respectively: 1) benzo[a]pyrene, 1') benzo[a]pyrene plus

* The limit value of the K region set by Pullman and Pullman [2] is a complex index equal to 3.31β , where β for the polycondensed aromatic hydrocarbons is about 20 kcal/mole.

UV light, 2) benzo[e]pyrene, 2') benzo[e]pyrene plus UV light, 3) pyrene, 3') pyrene plus UV light, 4) chrysene, 4') chrysene plus UV light, 5) only UV light. The painting was performed twice weekly by delivering a drop in the clipped interscapular area which was about 1 cm². The irradiations were carried out four times a week and two hours a day, using a 100 watt UV lamp with maximum output at 3660 Å and keeping each group of 20 mice in plastic containers 10 x 10 cm and 3 cm high, covered by a metallic net. The intensity of the UV light, delivered by a bulb lamp at 20 cm from the mice, was 8550 $\mu\text{W}/\text{cm}^2$. A fan system directed at the light beam regulated the temperature at 25° at the level of the mice. The experiment lasted 18 weeks. After that period all mice survived. No tumors were disclosed in the irradiated-only group or in the groups both irradiated and non-irradiated, treated with the non-carcinogenic chrysene (V), benzo[e]pyrene (VII) and pyrene (VIII). The group treated with the benzo[a]pyrene (I) showed 31% of tumors whereas the group with benzo[a]pyrene plus irradiation showed 63% of tumors. The positive photo-carcinogenic effect for I is in agreement with the previous results [5].

DISCUSSION

The benzo[a]pyrene in the photoproduct III is substituted at the 6-carbon atom, which is known from the literature to be the most reactive one. The same UV spectra for the photoproducts arising from irradiation of the benzo[a]pyrene and all bases of nucleic acids [3,4] or DNA [15] suggest that the same position of the hydrocarbon is bound to the biological substrates. Therefore, the 4,5-double bond (K region) does not play the major role in the photoreaction with these nucleic acid components.

The methylcytosine in III is substituted by the hydrocarbon at the 5-position, which has been postulated by theoretical calculations to be the most nucleophilic one, both in the free base and in the cytosine-guanine complementary pair [16]. Besides, the nucleophile hydroxylamine reacts specifically at the 6-position of cytosine [17]. Thus, the reactive intermediate of the hydrocarbon produced by UV irradiation presumably displays a positive charge localized on the meso-anthracenic position. In this connection, further compelling evidence stems from the radical cation of benzo[a]pyrene induced by chemical oxidation. This intermediate reacts at the 6-position with the nucleophilic nitrogen of pyridine [18].

In the biological experiment the data for benzo[a]pyrene confirm the previous results [5], namely, that there is an increase of cancer induction in the mice painted and irradiated as compared to the mice only painted with the same compound. If the extent of binding to the biological constituents accounts for the photodynamic action of benzo[a]pyrene, the K region is not responsible for this effect since the most active position is the 6-carbon atom, as above described for the photochemical reaction between the hydrocarbon and methylcytosine or other nucleic acid components.

The absence of tumors in mice treated with benzo[e]pyrene, pyrene, or chrysene, and then irradiated is another bit of evidence against the biological importance of the K region in triggering the cancer process. It is worthwhile to observe in this connection the photo-carcinogenic activity of the non-carcinogenic anthracene on painted and irradiated mice [19]. The high chemical as well as photochemical [20] reactivity of this hydrocarbon at the meso-positions is well known.

The precise way in which the very active 6-position of the benzo[a]-pyrene might be related to the carcinogenic activity of the hydrocarbon

is beyond the scope of this paper. The interested reader will find that matter presented elsewhere [21].

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FIGURE LEGENDS

Figure 1. Scheme of the photochemical coupling.

Figure 2. The infrared spectrum in chloroform of the 6-(1-methylcytos-5-yl)-benzo[a]pyrene.

Figure 3. The ultraviolet spectra in 95% ethanol of benzo[a]pyrene (A), 6-methylbenzo[a]pyrene (B), and 6-(methylcytos-5-yl)-benzo[a]pyrene (C).

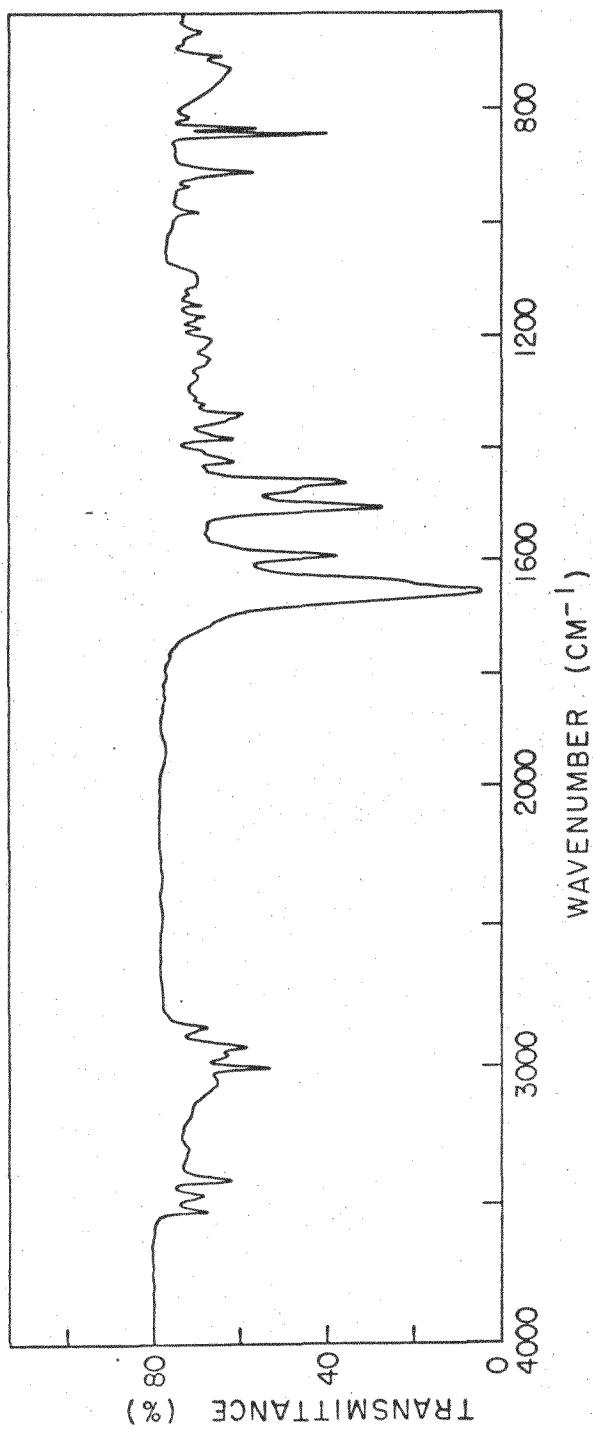
Figure 4. The ultraviolet absorption spectra of 4,5-diacetoxy-4,5-dihydro-benzo[a]pyrene (A) and chrysene (B) in 95% ethanol.

Figure 5A. The 220 MHz NMR spectrum of 1-methylcytosine (2% wt/v) in dimethyl- d_6 -sulfoxide. The scale is referred to TMS as internal standard.

Figure 5B. The 220 MHz spectrum of 6-(1-methylcytos-5-yl)-benzo[a]pyrene (0.5 - 1% wt/v) in saturated solution of chloroform-d (isotopic purity 100%) at 17°. The scale is referred to TMS as internal standard.

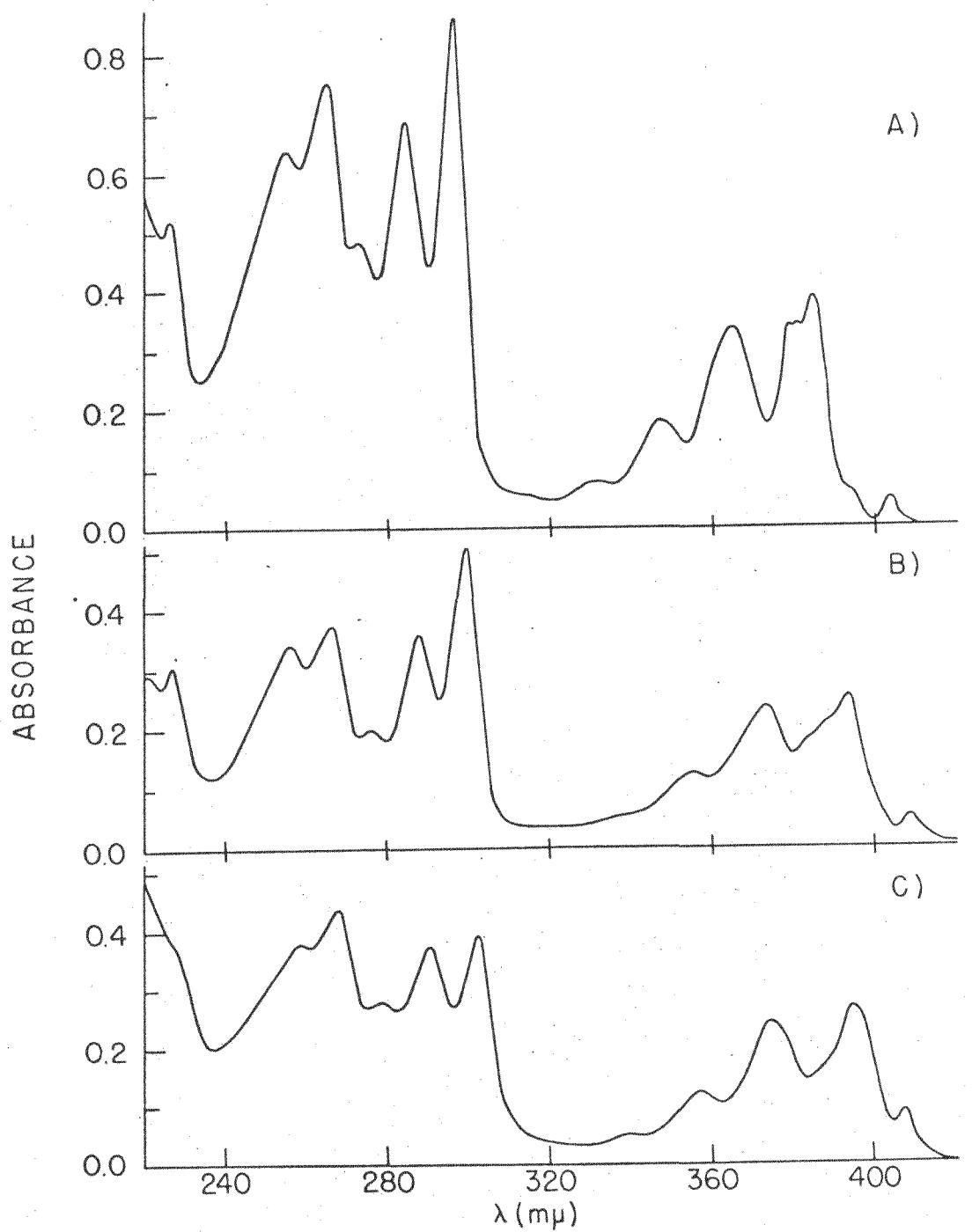
Figure 6A. The 220 MHz NMR expanded spectrum of 6-(1-methylcytos-5-yl)-benzo[a]pyrene. The coupling constants are in H_z and have been determined by expansion at 2 H_z per cm. The accuracy of the coupling constant measurements is $\pm 0.2 H_z$.

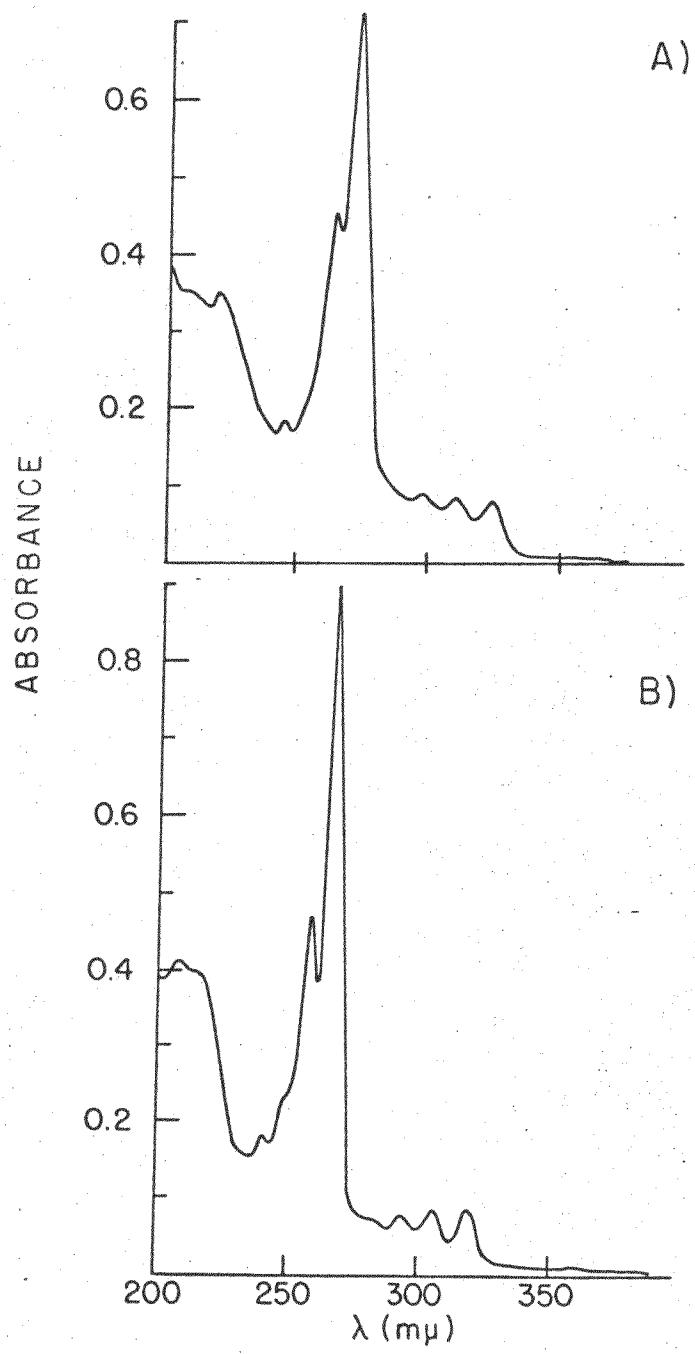
Figure 6B. The 220 MHz NMR spectrum of benzo[a]pyrene (2% wt/v) in $CDCl_3$ at 17°. The scale is referred to TMS as internal standard. The coupling constants are in H_z and have been determined by expansion at 2 H_z per cm. The accuracy of the coupling constant measurements is $\pm 0.1 H_z$.

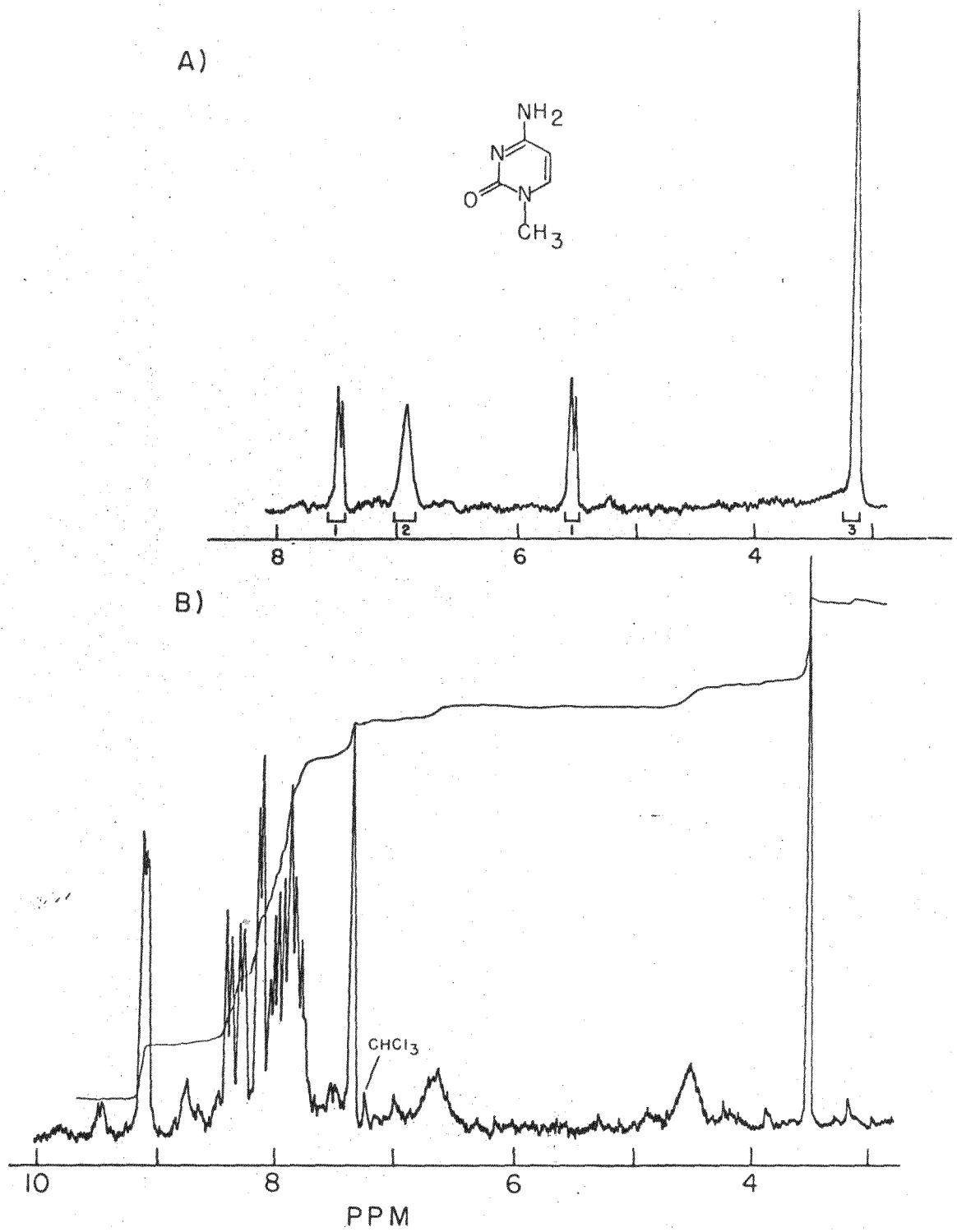


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Figure 2

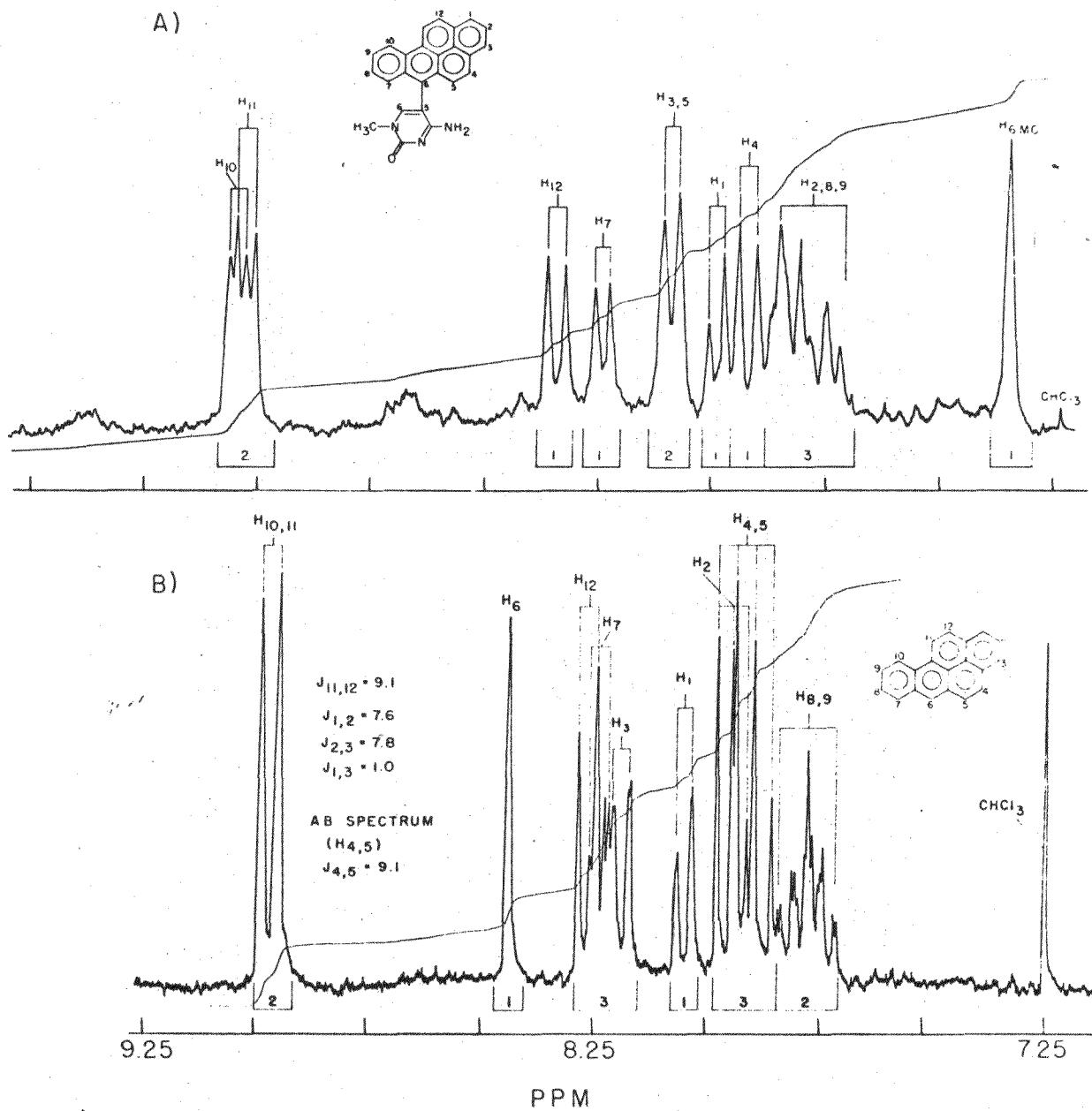




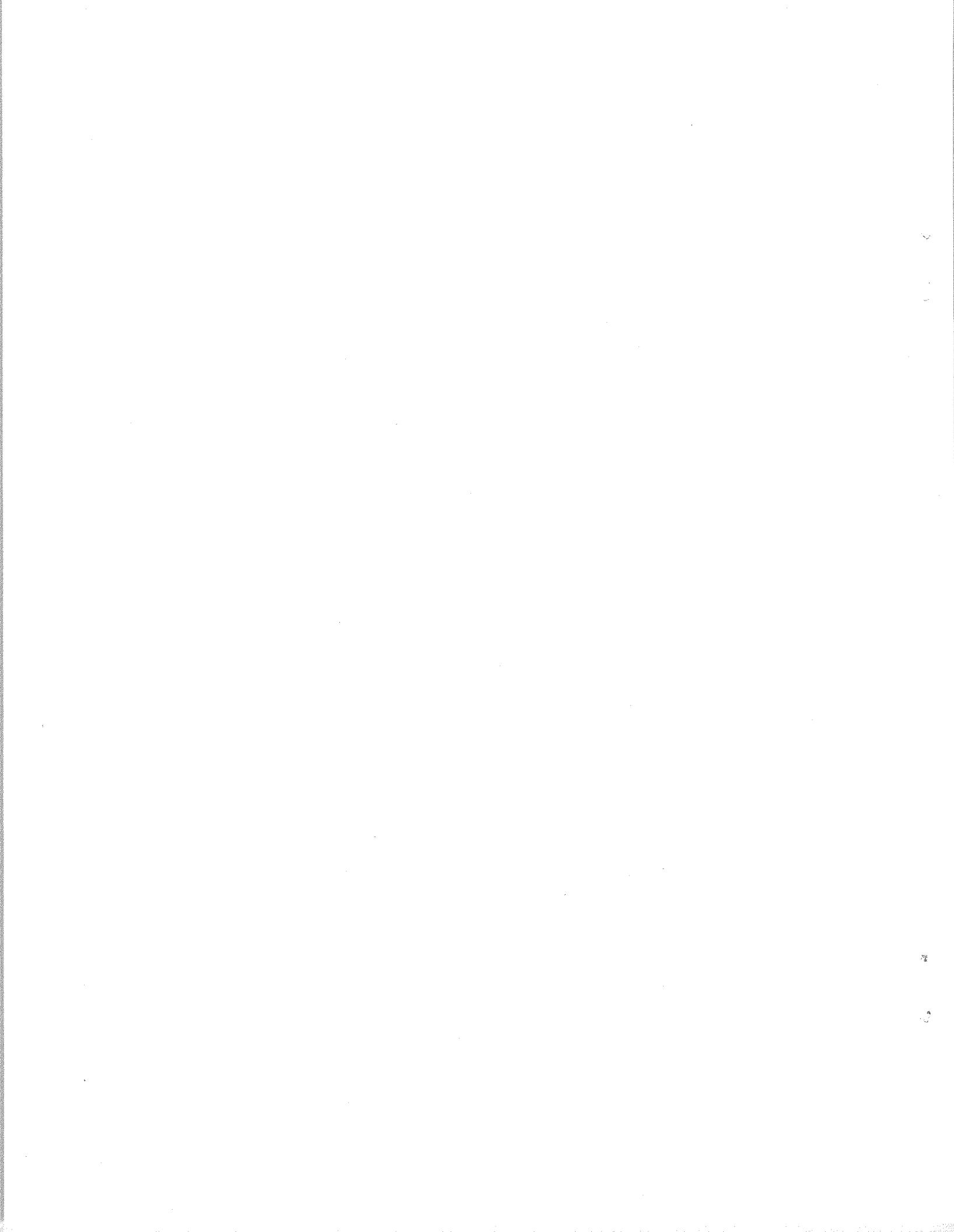


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Figure 5



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Figure 6



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