

Photoproducts from DNA pyrimidine bases and polycyclic aromatic hydrocarbons

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ABSTRACT

The major photoproduct formed between benzo[a]pyrene and thymine is identified as 1-(benzo[a]pyren-6-yl)-thymine by means of spectroscopic analysis and isotopic syntheses. Irradiation of 1-methylcytosine hydrochloride and anthracene gives two isolable photoproducts of which one is assigned the structure 5-(anthracen-9-yl)-1-methylcytosine.

INTRODUCTION

Benzo[a]pyrene and other polycyclic aromatic hydrocarbons bind covalently to DNA *in vitro* and *in vivo* and this phenomenon is widely believed to be associated with their carcinogenic characteristics.¹ The principal metabolic pathway in liver tissue involves oxidation by a mixed function cytochromal oxidase and gives phenols, quinones, and epoxides and hydrolysis products derived from the latter.² Recently, this last pattern of metabolic activation has been shown to lead directly to alkylation of RNA on the primary amino groups of guanosine residues³ and is linked to mutational phenomena in *Salmonellae* spp.⁴

The binding of a wide variety of polycyclic aromatic hydrocarbons to DNA can also be achieved by chemical and photochemical means⁵ and the effect of ultraviolet irradiation of mouse skin has been shown to enhance the induction of tumours by a defined concentration of benzo[a]pyrene^{6,7} and also to initiate tumour formation in the presence of anthracene.⁸ While this result was originally assigned to a photosensitising effect of the aromatic hydrocarbon⁸ more recently it has been attributed to photochemical binding of hydrocarbon to DNA *in vivo*.⁹

The photochemical coupling of benzo[a]pyrene with 1-methylcytosine⁷ and with thymine¹⁰ has been recognised to be oxidative in character and to involve aromatic substitution at position-6 in the benzo[a]pyrene molecule. We now report results of spectroscopic analysis and isotopic syntheses on the structure of the major benzo[a]pyrene:thymine photoproduct and also results

of experiments on the coupling of anthracene to 1-methylcytosine.

EXPERIMENTAL

Benzo[a]pyrene : Thymine Photoproduct. - A solution of thymine (657 mg) in distilled water (200 ml) was added to a solution of benzo[a]pyrene (19.6 mg) in AnalaR grade acetone (200 ml) and the clear solution, of violet fluorescence, kept overnight at 0° in the dark. The solution was then purged with pure nitrogen in a 2 l volumetric flask and irradiated for 44 hr at room temperature under nitrogen by means of a Philips 125 W 'black light' lamp located 20 cm below the base of the flask. The acetone was evaporated in vacuo and the aqueous suspension extracted with benzene (3 x 250 ml) and chloroform (7 x 350 ml). The combined extracts were dried (MgSO₄) and evaporated to a brown solution (2 ml) which was applied to a single alumina t.l.c. plate (20 x 20 cm) and developed with benzene:chloroform (1:1 v/v). The band at the origin was collected and extracted continuously for 3 hr with methanol. The extract was concentrated and applied to two silica t.l.c. plates (20 x 20 cm) which were developed in acetone:benzene (2:3 v/v).

Four fluorescent bands were detected under u.v. light. The most prominent, R_F 0.75, was collected and eluted with methanol to give the *product* in a spectroscopic yield of about 10% based on unrecovered hydrocarbon. Combination of this fraction from three similar experiments followed by rechromatography gave the *product* (2.7 mg, 3%) as a pale yellow crystalline solid, *d* 300°C. It was homogeneous on t.l.c. in several solvent systems and corresponded in all respects to the material described previously.¹⁰ Its n.m.r. spectrum was recorded in CDCl₃ at 220 MHz on a Perkin Elmer R34 spectrometer (Figure 1) and high resolution mass spectral data are presented in the following Table. Ultraviolet absorption and emission data have been

| M/e observed | Formula | M/e calcd. | 10 ³ x Error |
|--------------|---|------------|-------------------------|
| 376.1220 | C ₂₅ H ₁₆ N ₂ O ₂ | 376.1211 | 0.91 |
| 333.1143 | C ₂₄ H ₁₅ NO | 333.1153 | -0.97 |
| 305.1186 | C ₂₃ H ₁₅ N | 305.1204 | -1.77 |
| 304.1126 | C ₂₃ H ₁₄ N | 304.1125 | 0.12 |
| 290.0963 | C ₂₂ H ₁₂ N | 290.0969 | -0.61 |
| 277.0867 | C ₂₁ H ₁₁ N | 277.0891 | -2.31 |

described elsewhere.¹⁰

The minor, slower-migrating photoproduct described previously was not found as a component of all reactions and no attempt was made to characterise it further.¹⁰

6-Tritiobenzo[a]pyrene. - 6-Iodobenzo[a]pyrene¹¹ (200 mg, 0.53 mmol) in dry tetrahydrofuran (50 ml) was added to a solution of butyl lithium (1.5 mmol) in dry tetrahydrofuran (10 ml) at -78°C. After 30 min HTO was added (250 μ Ci, 50 μ l) and followed after 5 min stirring by D₂O (1 ml). The resulting mixture was brought to room temperature, washed with dil. HCl and water, dried (MgSO₄) and evaporated. The residual hydrocarbon was purified by t.l.c. on silica plates developed with cyclohexane:benzene (9:1 v/v). The product was obtained as yellow needles from benzene:isopropanol m.p. 176-178° (83 mg, 63%). The specific activity of this material was 30 mCi/mmol and conversion of a portion of it into 6-acetylbenzo[a]pyrene showed that 95% of the tritium was located at position-6.

Photoproduct from Thymine and 6-Tritiobenzo[a]pyrene. - 6-Tritiobenzo[a]pyrene (33 μ Ci) and carrier hydrocarbon (10 mg) were copurified by t.l.c. on silica and then dissolved in acetone (150 ml). Aliquots (0.1 ml) were removed for radiochemical assay using a Packard Tricarb 3383 liquid scintillation counter and had a specific activity of 1.10 μ Ci/ μ mol. The bulk of this solution was admixed with thymine (150 mg) in water (150 ml) and transformed into the photoproduct as before. Unreacted benzo[a]pyrene recovered from the alumina t.l.c. plate had a specific activity of 1.30 μ Ci/ μ mol). The photoproduct (6% yield) was characterised by absorption spectroscopy and by t.l.c. behaviour and had a specific radioactivity of 0.05 μ Ci/ μ mol.

Photoproduct from 6-Deuteriothymine and Benzo[a]pyrene. - 6-Deuteriothymine was prepared by decarboxylation of orotic acid (5 g, Sigma Chemicals) after equilibration in D₂O.¹² The product (670 mg, 18%) showed an isotopic purity of 80% gauged by mass spectrometry and by n.m.r. spectroscopy and was identical with thymine in all other respects. From this material (300 mg) and benzo[a]pyrene (9.6 mg) the corresponding photoproduct was prepared as before (4%) and characterised by low resolution mass spectrometry, M/e 378(32), 377(100), 376(28), 334(18), and 333(5%).

Photoproduct from 1-Methylcytosine and Anthracene. - A suspension of pure anthracene (1.6 g) in methanol (285 ml) and cyclohexanone (15 ml) was purged with nitrogen and stirred in a 500 ml flask containing an immersion type medium-pressure mercury lamp fitted with a Pyrex filter. A solution of 1-methylcytosine hydrochloride (100 mg) in methanol (100 ml) was added dropwise with continuous irradiation during 3.5 hr and irradiation continued for a further 26 hr at 25°C. A residual solid suspension was collected by filtration and identified as anthracene admixed with anthracene photodimer. The filtrate was evaporated *in vacuo* and freed from cyclohexanone by repeated

evaporation from petrol. The solid residue was extracted with cyclohexane (2 x 200 ml), dissolved in methanol (5 ml), and chromatographed on preparative alumina plates (20 x 46 cm) using acetone:benzene:water (7:2:1 v/v). Two fluorescent bands, R_F 0.64 and 0.76, were collected and extracted with methanol; several additional minor bands yielded insufficient material for further study.

The faster-migrating product A (0.5 mg) did not melt below 300°C, λ_{\max} (log ϵ) in alcohol 253 nm (ca. 5.0), 351, 368 (ca. 3.7) and 368 (ca. 3.7). Low resolution mass spectral data is given in Figure 2.

The slower-migrating product had m.p. 155-160°C, λ_{\max} (MeOH) 256 nm shifting to 251 nm on acidification with HCl during 2 min with doubling of extinction coefficient (to ca. 10^5) and weak bands at 372 and 393 nm; M/e 356(2.5), 355(6.5), 354(20), 208(15), 180(20), 170(30), and 178(100%).

RESULTS AND DISCUSSION

Benzo[a]pyrene:Thymine Photoproduct

The mass spectrometric and light absorption data both show that the photoproduct results from an oxidative linkage between benzopyrene (1) and thymine.¹⁰ The 220 MHz n.m.r. data (Figure 1) reveal the presence of the thymine H-6' at 7.20 δ somewhat broadened by weak coupling ($J = 0.8$ Hz) to the three-proton signal for the C-CH₃ group at 2.065 δ . The well-defined singlet for H-6 in the spectrum of benzo[a]pyrene¹³ is absent.¹⁴ The eleven

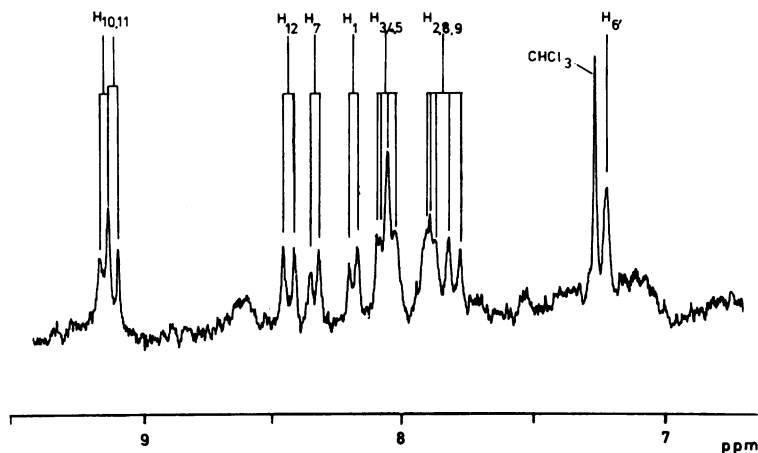
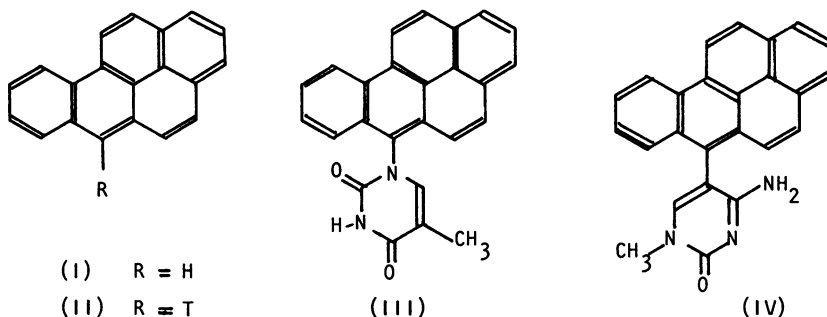


FIGURE 1. The 220 MHz expanded nmr spectrum for 1-(benzo[a]pyren-6-yl)-thymine at 30°C in $CDCl_3$ relative to TMS as internal standard. A 3-proton doublet ($J=0.8$ Hz) at 2.065 δ is present at high-field (not shown).

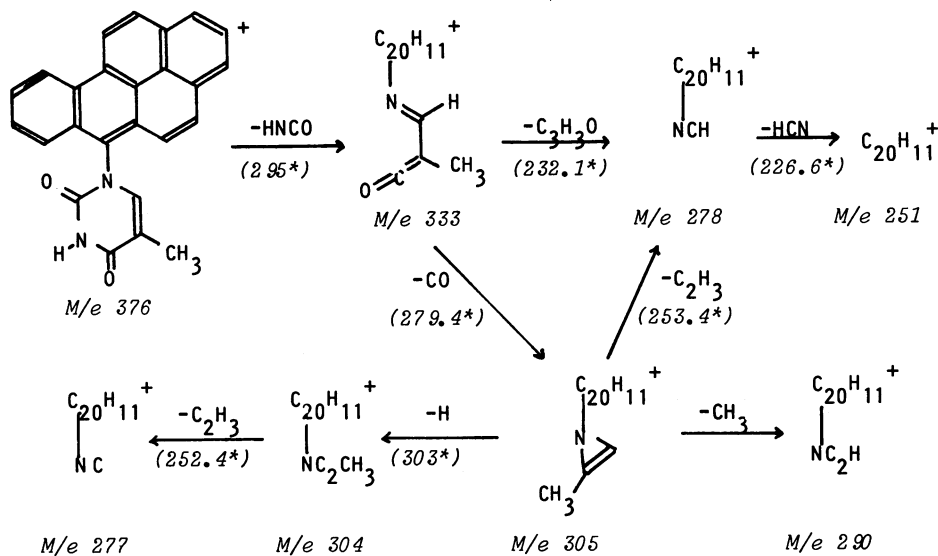
aromatic hydrogen signals are allocated in relation to the assignments for benzo[a]pyrene¹³ and for 5-(benzo[a]pyren-6-yl)-1-methylcytosine⁷ (IV). This spectroscopic evidence strongly indicates that thymine is bonded to position -6 of the aromatic system. Confirmation of this feature is provided by the preparation of the photoproduct from benzo[a]pyrene specifically tritiated at position-6 (II). Since the photoproduct (III) thus prepared shows the loss of 95% of the tritium present in (II), the remaining question concerns the position of attachment of the aromatic hydrocarbon to the thymine ring.



Preliminary data obtained by low resolution measurements of the mass spectrum of the photoproduct suggested the successive loss of two HNC₂O fragments. This was interpreted¹⁰ in favour of substitution at position-6 of the thymine ring. The high resolution data (Table) invalidate that analysis and, partly as a result of a rich abundance of metastable peaks, support the detailed fragmentation pattern given in the following Scheme, consistent with structure (III). This scheme reveals that HN(3)C(2)O is lost in the retro-Diels-Alder fashion characteristic of thymine derivatives¹⁵ and other fragmentations correspond to loss of CO and of the methyl group. Thus, the benzo[a]pyrenyl group is bonded to either N-1 or C-6 of thymine.

This choice was resolved in two ways. First, the 220 MHz n.m.r. spectrum clearly shows the presence of the thymine H-6 at 7.205δ weakly coupled ($J = 0.8$ Hz) to the thymine methyl signal at 2.065δ. Secondly, the synthesis of the photoproduct from 6-deuteriothymine¹² afforded the isotopic derivative of (III) for which the parent ion and principal fragment ion showed a unit mass increment.

This evidence along with the ultraviolet absorption and emission data previously presented¹⁰ fully support the assignment of structure (III) for the benzo[a]pyrene:thymine photoproduct. It may be presumed that the isomeric substance encountered in our preliminary work¹⁰ has the benzo[a]pyrenyl group



SCHEME. Mass Spectrometric fragmentation pattern for (III). Figures in parentheses indicate metastable ions corresponding to the fragmentation indicated.

attached to N-3 of thymine since we have repeatedly failed to obtain a benzo[a]pyrene photoproduct from 1,3-dimethylthymine.

Anthracene:1-Methylcytosine Photoproduct

The ready transformation of anthracene into its photodimer¹⁶ makes difficult the isolation of photoproducts with other molecules. No photoproducts could be isolated from thymine or dimethylthymine with anthracene or 9,10-dimethylantracene in sensitised or unsensitised reactions. With 1-methylcytosine only trace amounts of photoproducts could be detected using 9,10-dimethylantracene and irradiation with anthracene at wavelengths above 350 nm resulted in photosensitised pyrimidine decomposition. Photosensitisation with acetone, which is known to form a photoproduct with 1-methylcytosine,¹⁷ resulted in rapid degradation of the pyrimidine but photosensitisation using Pyrex-filtered radiation and cyclohexanone led to the formation of a variety of anthracene:1-methylcytosine photoproducts of which two could be isolated by t.l.c. in very low yield.

Product A was t.l.c. homogeneous and had light absorption and emission spectra characteristic of 9-alkylantracenes.¹⁸ Attempts to observe its n.m.r. spectrum were frustrated by the formation of traces of anthraquinone in solutions of the photoproduct. Low resolution mass spectrometry of this

material (Fig. 2) showed a parent ion at m/e 301 corresponding to oxidative linkage of anthracene to 1-methylcytosine. The base peak of the spectrum at m/e 178 corresponds to anthracene and the principal fragmentations to m/e 258 and 217 to successive loss of HNCO and CH_3NC respectively, characteristic features of cytosine mass spectra.¹⁹ Though limited, these data and the mechanistic analogy to the formation of (IV) clearly support the designation of this photoproduct as 5-(anthracen-9-yl)-1-methylcytosine (V).

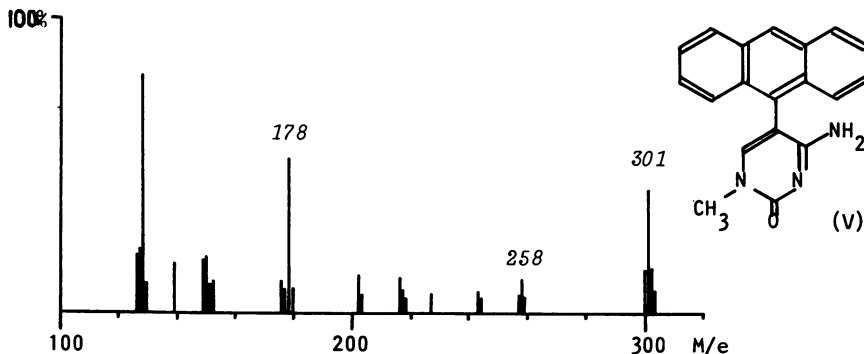


FIGURE 2. Mass spectrum of anthracene:1-methylcytosine photoproduct (V).

Photoproduct B was obtained in trace amounts only and is both less stable and more complex than photoproduct A. It appears to be a 2:1 photoproduct of anthracene and 1-methylcytosine which gives bianthryl on electron impact and contains one anthracene and one dihydroanthracene chromophore. The facile generation of traces of anthraquinone from it made further analysis impracticable.

CONCLUSION

It is evident that photochemical combination of pyrimidine bases with polycyclic aromatic hydrocarbons is an inefficient process which, in model systems, competes poorly with self-association of hydrocarbon and pyrimidine species. It is likely that in the presence of DNA the situation is simplified by the dispersion of hydrocarbon molecules as a result of the detergent-like effect of the nucleic acid.

The pattern of linkage of the hydrocarbon to pyrimidines shown in structures (III) - (V) is consistent with the observed loss of tritium on photochemical binding of benzo[a]pyrene²⁰ and anthracene²¹ to DNA *in vitro* and in tissue culture.⁹ It supports the suggestion that the photocarcinogenicity of these hydrocarbons is associated with oxidative bonding between them and DNA bases to cause a potentially mutagenic lesion.

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