Tetrahedron Letters No. 7, pp 589 - 592, 1972. Pergamon Press. Printed in Great Britain.

THE STRUCTURE OF THE THYMINE:3,4-BENZOPYRENE PHOTOPRODUCT

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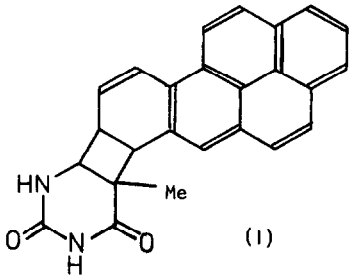
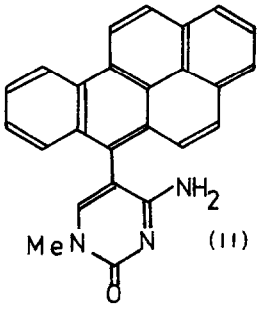
(Received in UK 3 January 1972; accepted for publication 13 January 1972)

Many chemical carcinogens have been shown to alkylate the heterocyclic bases of DNA and RNA and in some cases a correlation has been observed between such alkylation and carcinogenic activity.[1] Although it is well established that the polycyclic aromatic hydrocarbons can be linked covalently to DNA by several chemical means,[2-4] which are usually oxidative in character, the detailed mode of such linking has been difficult to establish. Rice[5] has suggested that a cyclobutane adduct is formed between cytosine and 3,4-benzopyrene involving the 5,6-double bond of the pyrimidine and the K-region of the hydrocarbon - a proposal which implies a similarity to the well-known reaction of DNA on ultraviolet irradiation to form cyclobutane pyrimidine dimers.[6] Antonello, on the other hand, proposed[7] a structure (I) for a photoproduct of thymine and 3,4-benzopyrene in which the four-membered ring is joined at positions 7 and 8 of the hydrocarbon.

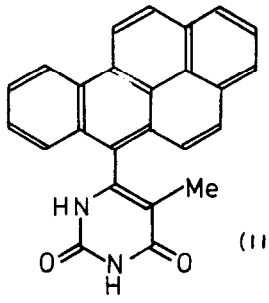
In contrast, Cavalieri and Calvin[8] have recently demonstrated that 1-methylcytosine becomes attached by a single bond to the 6-position of 3,4-benzopyrene on irradiation at 350nm and gives the product (II) an oxidative process. We wish to report our evidence for a similar structure (III) for the photoproduct of thymine and 3,4-benzopyrene.



(I)



(II)



(III)

The crude photoproduct was obtained with small modification of Antonello's method[7] and was separated from 3,4-benzopyrene and its quinones by preparative thin-layer chromatography. Two solid fractions were obtained, the major exhibited a blue fluorescence and the minor showed a greenish-blue emission. Both compounds were purified by repeated t.l.c. and obtained as pale yellow solids, neither melting below 300°. Attempted sublimation of either material failed to give further purification but rather resulted in decomposition of the photoproducts to an oily sublimate and an involatile residue. However, both the major and minor products afforded satisfactory mass spectra with parent ions at M/e 376 and markedly similar fragmentation patterns. Only the more mobile compound was obtained in sufficient abundance (3% yield based on 3,4-benzopyrene) for full identification.

The ultraviolet absorption spectrum of this product (Figure 1) shows a remarkable

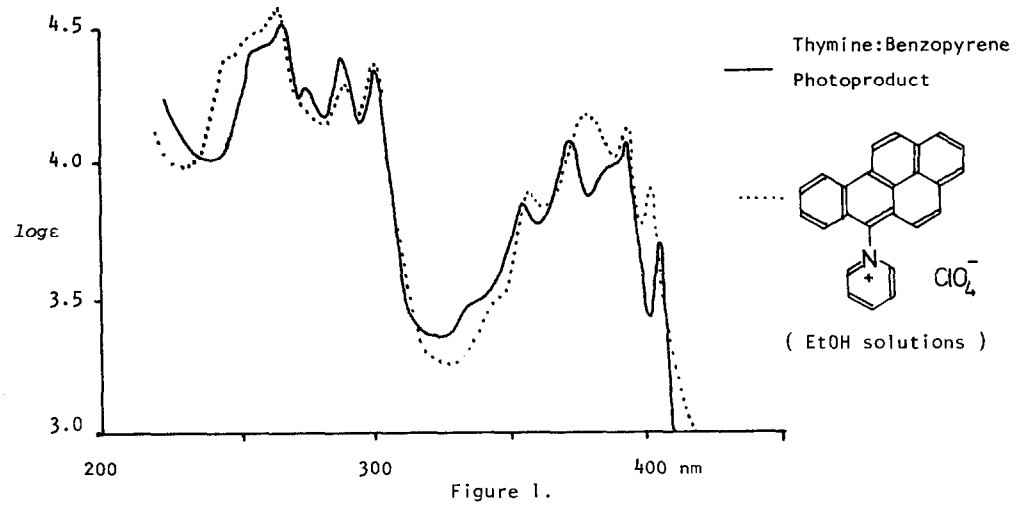


Figure 1.

similarity to that of Rochlitz's *N*-(6-benzopyrenyl)-pyridinium perchlorate.[9] Both of them display bathochromic shifts of about 10nm from the principal absorption bands of 3,4-benzopyrene whilst retaining all the features of its fine structure. This provides good evidence that the π-electron system of the polycyclic hydrocarbon is intact in both major and minor photoproducts since they have very similar absorption spectra.

The fluorescence spectrum of the major isomer is characterised by strong emission at 411 (100%) 435(62%), and 462(19%)nm with excitation at 380, 335, 310nm and shorter wavelengths.

It is very similar to the fluorescence spectrum of 3,4-benzopyrene and also shows behaviour characteristic of a single fluorescent species.

High resolution mass spectrometry established the molecular formula of $C_{25}H_{16}N_2O_2$ for the parent ion (Found M/e 376.1209; requires M/e 376.1212) while the low resolution spectrum (Figure 2) shows well-defined fragmentations down to M/e 252, apparently the 3,4-benzopyrene cation. The loss of HNCO (P-43) followed by CO (P-71) is characteristic of thymine ring fragmentation[10] while the peak at M/e 290 corresponds to the loss of two HNCO units and demonstrates that the hydrocarbon radical is not linked to the pyrimidine ring *via* either nitrogen or oxygen. It must therefore be attached through carbon-6 or the exocyclic methyl group, both positions known to be reactive towards radical reagents.[11]

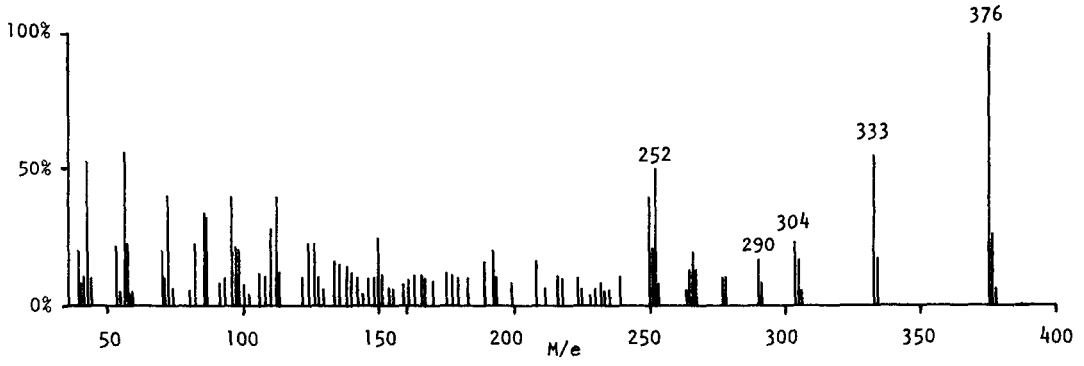


Figure 2. Mass Spectrum of Thymine:Benzopyrene Photoproduct

The position of attachment of the thymine residue to the benzopyrene is clarified by the proton magnetic resonance spectrum of the major product (c.a.t. on 280 scans at 100MHz in $CDCl_3$). This shows an 11-proton complex signal at 7.5-9.1δ, two N-H protons at 4.3δ, and three protons at 3.70δ attributed to the thymine methyl group. Detailed analysis of the aromatic region of the spectrum revealed that the signal attributed to hydrogen-6 of the 3,4-benzopyrene moiety, which is totally absent in the spectrum of the 1-methylcytosine:benzopyrene product,[8] is not entirely lost but that some 20% of its intensity remained with a complementary diminution in the signal associated with positions 12 and 1.

The above evidence establishes that the 3,4-benzopyrene is joined through a single covalent bond to position-6 of the thymine ring and that it fully retains its aromatic character.

Although the major photoproduct appears to be homogeneous, as gauged by its behaviour on t.l.c., the p.m.r. evidence suggests that it may be contaminated with some 20% of an isomeric material while the remaining spectroscopic data suggest that the minor photoproduct is also isomeric with the major. In particular, the coincidence of fluorescence of the major and minor isomers is consistent with their being positional isomers with respect to substitution on the benzopyrene residue.

We therefore conclude that the major photoproduct has thymine linked to position-6 of the 3,4-benzopyrene (III) and is, as yet, inseparable from an isomer linked probably to position 1 or 12 of the hydrocarbon.

It may well be significant that the SCF MO calculations of Dewar[12] predict that the radical cation of 3,4-benzopyrene should exhibit charge localisation in the order position $6 \gg 1 > 12 \geq 3$. Moreover, the recognition that more than one position in 3,4-benzopyrene is available for attachment to thymine raises the possibility that such a hydrocarbon may be able to alkylate bases in both strands of DNA by the same molecule and thus effect cross-linking of the complementary DNA strands.

Acknowledgement This work was supported by a Grant from the Medical Research Council.

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