Structure of a 7,12-Dimethylbenz[*a*]anthracene 5,6-Oxide Derivative bound to C-8 of Guanosine

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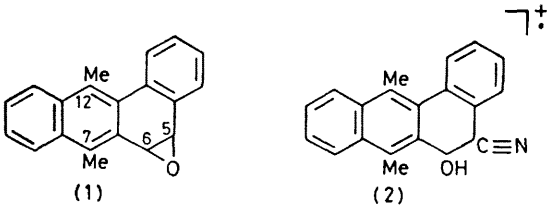
Summary The structure of one of the RNA adducts formed when guanosine reacts with (±)-7,12-dimethylbenz[*a*]anthracene 5,6-oxide under alkaline conditions has been determined as represented by (**3**).

STUDIES with mouse skin and hamster or mouse embryo cells indicate that the major binding of 7,12-dimethylbenz-[*a*]anthracene (DMBA) to DNA involves generation of a diol–epoxide at C-1 to C-4 ('bay region') of DMBA;[1–6] however, so far none of the structures of these adducts have been elucidated. However, recent experiments with 5-fluoro-DMBA implicate the involvement of C-5 in metabolic activation[7] into a mutagen or carcinogen, although this is probably not the major route.



Treatment of (±)-DMBA 5,6-oxide (**1**) in 50% aqueous acetone with polyguanylic acid at pH 5—6 gives four adducts in which the N^2 of guanosine is attached to C-5 (α and β) and C-6 (α and β).[8] However, since none of them corresponded to the mouse or hamster *in vivo* products,[3] another set of *in vitro* products was prepared by treating DMBA 5,6-oxide with guanosine in a 1:2 mixture of aqueous NaOH and acetone (pH 9·5) for 4 days at 37 °C. *Ca.* 15% of the guanosine reacted to give products G*-(Ia), (Ib), and (II)—(V). Of the six products, G*-(Ia), (Ib), and (II) corresponded to the adducts isolated from the RNA of rat liver cells treated with [^{3}H]DMBA in culture; however, this constituted < 10% of the total RNA [^{3}H]-DMBA adducts.[9] Furthermore, we have shown that G*-(Ia) and G*-(Ib) are, respectively, (5*S*)-guanosyl-(6*R*)-hydroxy- and (6*R*)-guanosyl-(5*S*)-hydroxy-adducts resulting from attacks of the guanosyl 2′-OH at C-5 and C-6 of the DMBA oxide with concomitant *trans* opening of the epoxide.[10] In this work we elucidate the structures of G*-(II) to (V) to show that G*-(II) is represented by structure (**3**).

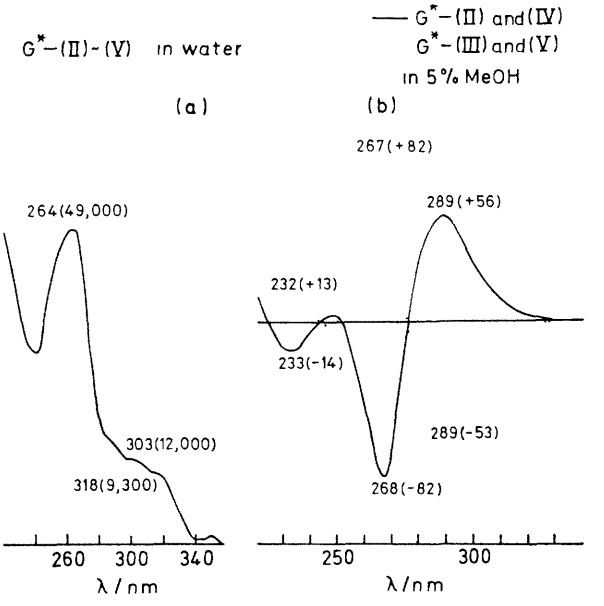
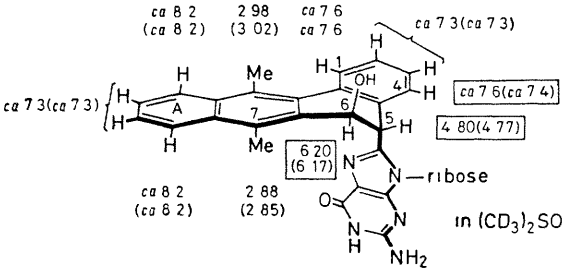


FIGURE. (a) U.v. spectra of G*-(II)–(V); numerals in parentheses denote ε values. (b) C.d. spectra of G*-(II)–(V); numerals in parentheses denote Δε values.

Structural studies were carried out on G*-(II) and (III), the two major adducts (*ca.* 0·5 mg each); data on G*-(IV) and (V) were used only in an auxiliary sense because of the minute quantities. The u.v. spectrum of G*-(II), shown in the Figure (a), was superimposable on those of the other three, whereas the c.d. spectra [Figure (b)] constituted two mirror image pairs. Hence the spatial relations between

the guanine and DMBA-derived units are almost identical in (II)/(IV) and in (III)/(V), the pairs are epimeric with respect to the chirality at the 5,6- positions of the benz-anthracene ring, but not with respect to the ribose unit Measurements of the c d spectrum under different pH's[10,11] showed the pK' values to be $<1{\cdot}0$ and 10·4 The presence of *two* pK values restricts the point of attachment of the DMBA unit to N², C-8, or the ribose residue of guanosine



220 MHz ¹H-n m r data of G*-(II) (**3**) and (III) (in parentheses) $(CD_3)_2SO$ The ribose peaks were all identified but are deleted from the drawing

The high-resolution electron impact mass spectrum (250 °C) of G*-(II) peracetate (formed *in situ* by micro-acetylation[12]) showed, in addition to peaks at 272 (9%, $C_{20}H_{16}O$, corresponds to DMBA mono-ol), 256 (100%, $C_{20}H_{16}$, DMBA), and 241 (90%, $C_{19}H_{13}$, DMBA minus methyl), a clear peak at 299 (2%) [12] This peak, corresponding to the fragment shown in (**2**), establishes that G*-(II) is linked through the guanosine C-8 The fact that the pK_1' of G*-(II), in contrast to the 2 2 value for guanosine, is lower than 1 is in accord with the attachment of a group at C-8 because this would favour deprotonation of the imidazole ring

The ¹H-n m r data of G*-(II) and (III) in $(CD_3)_2SO$, where the solvent peaks were removed by the inversion–recovery method,[13] are shown in structure (**3**) The following points were noted in the ¹H-n m r spectra (i) no singlet was present in the region of 7·0—8 5 p p m where the characteristic guanosine H-8 appears,[14] (ii) the naphthalenoid ring A protons constitute a typical AA'BB' symmetric pattern, (iii) all signals for (II) and (III) including the H-5, H-6, and 7-Me signals had identical or very similar chemical shifts except for H-4 which was centred around 7 6 p p m in G*-(II) (hence overlapping with H-2) and around 7 4 p p m in G*-(III) (an isolated 1 H multiplet)

Observation (iii) allows the linkage of guanosine to C-5 in both G*-(II) and (III) † Thus it is the DMBA H-4 which is affected by the guanosine configuration since, depending on whether this is α or β, the chiral ribose group exerts a different influence on H-4 The attack of guanosine at C-5 is also preferred on steric grounds because of the 7-Me group The antipodal relation between the chromophores in G*-(II) and (III) is corroborated by their c d spectra [Figure (b)].

The c d spectra of G*-(IV) and (V) are antipodal, and also superimposable on those of (II) and (III), respectively This shows that in adducts (IV) and (V) the guanosine C-8 must be linked to the DMBA through the same carbon, *i e*, C-5 ‡ Because (II)/(III) were the major pair and (IV)/(V) the minor, we conclude them to be the *trans*- and *cis*-cleavage products, respectively

In view of the fact that the other two tissue culture products G*-(Ia) and (Ib) both result from the nucleophilic attack of the ribose-2'-OH on DMBA (5*R*,6*S*)-oxide [or 'β'-epoxide when DMBA is drawn as in (**1**)] it is reasonable to assume that the third tissue culture product G*-(II) also results from the same 5,6-oxide The structure of G*-(II) can therefore be represented by (**3**) although there is currently no direct proof regarding its absolute configuration The structures of the three tissue culture products G*-(Ia), (Ib)[10] and (II), which are also formed upon treating DMBA 5,6-oxide with guanosine at pH *ca* 9 5 have thus been determined That the chemically produced adducts G*-(I)–(V) result from nucleophilic attacks of the guanosyl 2'-OH or C-8 can be accounted for by the facts that the 2'-OH is the most acidic of the ribosyl hydroxy groups[15] and that at pH 9 5 the N¹–H is 50% anionic (pK_2 of guanosine 9·5) so that C-8 becomes a nucleophilic centre

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† G*-(II) and (III) are not positional isomers since the ¹H n m r data (ref 8) for isomeric DMBA-guanosine N^2 adducts, DMBA 5,6-oxide and DMBA-5,6-diols show that H-6 is always located at 0 4—0 5 p p m lower field than H-5 in corresponding substitutions The difference is presumably due to the compression effect of the 7-Me group

‡ The complex c d spectra are highly characteristic and in a sense serve as a finger print Therefore, if the bond linking the DMBA and guanosine groups were not the same, it should not lead to superimposable c d spectra The quantities of G*-(IV) and (V) were insufficient for ¹H-n m r studies

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