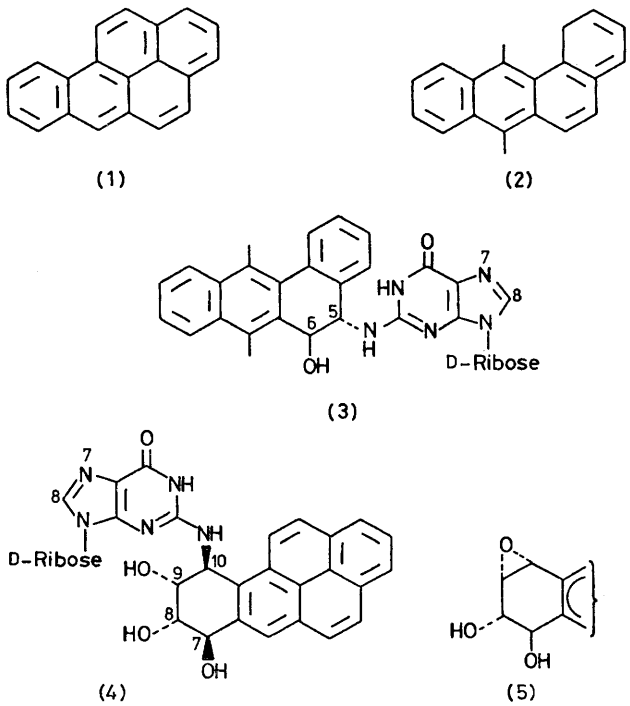
Two Micromethods for Determining the Linkage of Adducts formed between Polyaromatic Hydrocarbons and Nucleic Acid Bases

By H. KASAI and K. NAKANISHI*
(*Department of Chemistry, Columbia University, New York, New York* 10027)
and S. TRAIMAN
(*Hoffmann-La Roche Inc., Nutley, New Jersey* 07110)

Summary Two micromethods, one based on c.d.–pK measurements and the other on Fourier transform i.r. spectroscopy, for determining the linkage of adducts formed between polyaromatic hydrocarbons and nucleic acid bases are described; both methods can be performed on the microgram scale, in contrast to other spectroscopic methods which require larger quantities of sample.

THE covalent binding of polyaromatic hydrocarbons such as benzo[*a*]pyrene (**1**) and 7,12-dimethylbenz[*a*]anthracene (**2**) to RNA and DNA is considered to play an essential role in their carcinogenic actions.[1] Recently the structures of several adducts formed *in vitro* and by tissue culture have been fully elucidated. Thus adducts such as (**3**) are formed when the 5,6-epoxide of (**2**) reacts with polyguanylic acid,[2,3] and adduct (**4**) is formed when the tetrahydrodiol epoxide (**5**) of (**1**) reacts with polyguanylic acid[3,4] or when [^{3}H]-(**1**) is incubated with bovine[3,4] and human[5] bronchial explants;† adduct (**4**) with deoxyribose instead of ribose is produced from [^{3}H]-(**1**) and human bronchial DNA.† Adducts such as (**4**) and its stereo-isomers resulting from reaction of polyguanylic acid with the epoxide isomeric with (**5**) (9β,10β) and from exposure of mouse skin to [^{3}H]-(**1**) have been elucidated by Jerina, Koreeda, and their co-workers.[6]



Straub *et al.*[7] have indicated that the adducts formed from calf thymus DNA and racemic diol epoxide (**5**) involve binding through the exocyclic amino groups of deoxy-guanine, -adenosine, and -cytidine. The full structure determinations of two adducts resulting from (**2**) and rat liver tissues have been shown to involve rear attacks of the guanosine 2′-OH at C-5 and C-6 of the 5,6-oxide of (**2**) with *trans* oxiran cleavage.[8,9]

In all cases studied by us, the amount of adducts available has been at the most 1—2 mg. Because water or Me_2SO is usually the solvent used for ^{1}H n.m.r. measurements non-routine techniques such as removal of solvent peaks by partially relaxed Fourier transform at non-ambient temperatures[2,4b] have to be used to elucidate the structure of the hydrocarbon unit. Moreover, ^{1}H n.m.r. measurements lead to numerous other problems caused by scarcity of sample. Determination of the point of attachment of the nucleoside to the hydrocarbon imposes a further problem since several sites are possible. For example, guanosine (see **4**), which is generally the best nucleophile of the nucleic acid bases,[10] can be linked to the hydrocarbon *via* its 1-N, N^2, 6-O, 7-N, C-8, or the ribose unit. Thus ^{1}H n.m.r. spectra usually do not lead to any definite conclusion regarding the substitution site on the nucleoside.

We here describe two convenient microspectroscopic methods which can be readily carried out on a microgram scale to determine the site of linkage on the base unit. This is exemplified by the two adducts (**3**) and (**4**).

*C.d.–p*K: the number and values of pK's are dependent on the substitution pattern of guanosine derivatives: guanosine 2·2 and 9·4, 1-Me-guanosine 2·6, 2-Me-guanosine 2·3 and 9·7, 6-Me-guanosine 2·4, and 7-Me-guanosine 7·1.[11]‡ The dissociation constants cannot be measured by titration, owing to the limited quantity of sample available, or by u.v. spectroscopy, owing to the domination of spectra by the strong non-dissociating polyaromatic hydrocarbon chromophore. Conveniently, however, the c.d. curves, which require solutions less dilute than for u.v. measurements, are complex and consist of several intense extrema because of the coupled interaction between the hydrocarbon and guanine chromophores. Thus although the c.d. curves have extrema at wavelengths corresponding to the hydro-

† Structure (**4**) represents the absolute configuration of the major tissue culture products.

‡ Koreeda, *et al.* have employed a method for estimating the pK_a' values by plotting changes in partition coefficients against pH values: Ref. 6a.

carbon absorption maxima, the extrema are the result of spatial interactions and therefore are subject to changes in the guanosine charge.

A plot of c.d. against pH therefore leads to measurements of pK_a' values as exemplified for adduct (**4**) in Figures 1 and 2; 10—20 μg were used in these measurements. Apparent dissociation constants of 2·1 and 9·1 (in 10% aq. MeOH) were obtained when the changes of $\Delta\epsilon$ at the 250 nm extrema ($\Delta\epsilon$ 72 at pH 7·1) were plotted. Similarly, for adduct (**3**) the pK_a' values were 2·4 and 9·3 (in 19% aq. MeOH); the peak at 267 nm, $\Delta\epsilon$ 37 (pH 7·5), was employed in this case. The method has been used recently in structural studies of various tissue culture and *in vitro* products.§

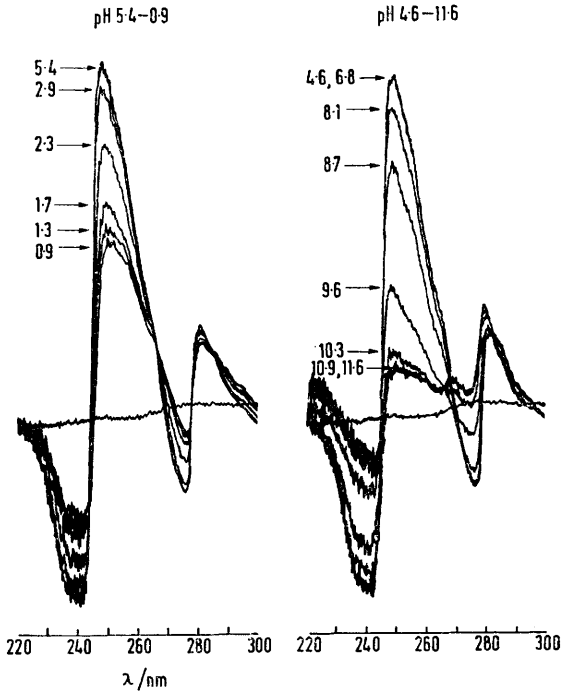


FIGURE 1. Change in c.d. of adduct (**4**) with pH, in 10% aq. MeOH. Because of the instability of (**4**) to acidic conditions, the initial solution (pH 4·6) was first raised to pH 11·6, the solution was neutralized to pH 5·4 and then acidified to pH 0·9. A JASCO J-40 instrument was used.

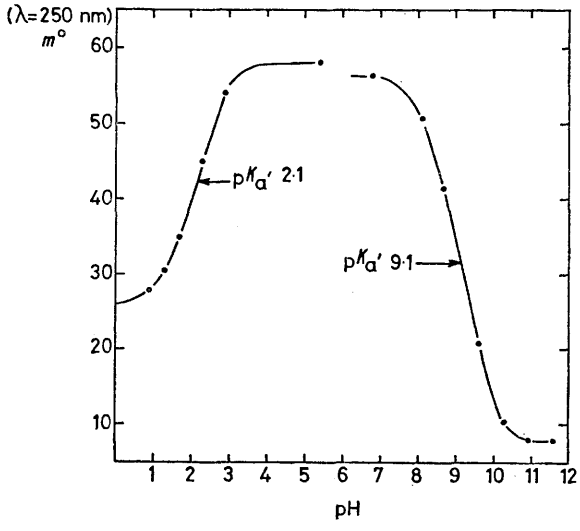


FIGURE 2. Plot of change in c.d. $\Delta\epsilon_{250}$ with pH for adduct (**4**) (see Figure 1).

Fourier transform i.r. spectroscopy: the idea was to measure the i.r. spectrum of the adduct in an appropriate solvent,¶ computer-subtract from the i.r. spectrum of the adduct that of the solvent and then a suitable derivative of the polyaromatic hydrocarbon, and to compare the residual spectrum with the spectra of authentic guanosines derivatives substituted at various positions. For instance, with the adduct (**3**) of (**2**) we expected the i.r. spectrum resulting from the operation: (**3**) — MeOH — (**2**)-dihydro-5,6-diol [5-OH instead of guanosine in (**3**)] to be similar to the i.r. spectrum of an authentic 2-substituted-guanosine. However, this technically cumbersome operation carried out initially proved to be unnecessary, as practically all prominent i.r. bands were due to the more polar guanosine unit both in the fingerprint as well as in the 1800—1400 cm^{-1} region, and the hydrocarbon unit exerted only minor influences.

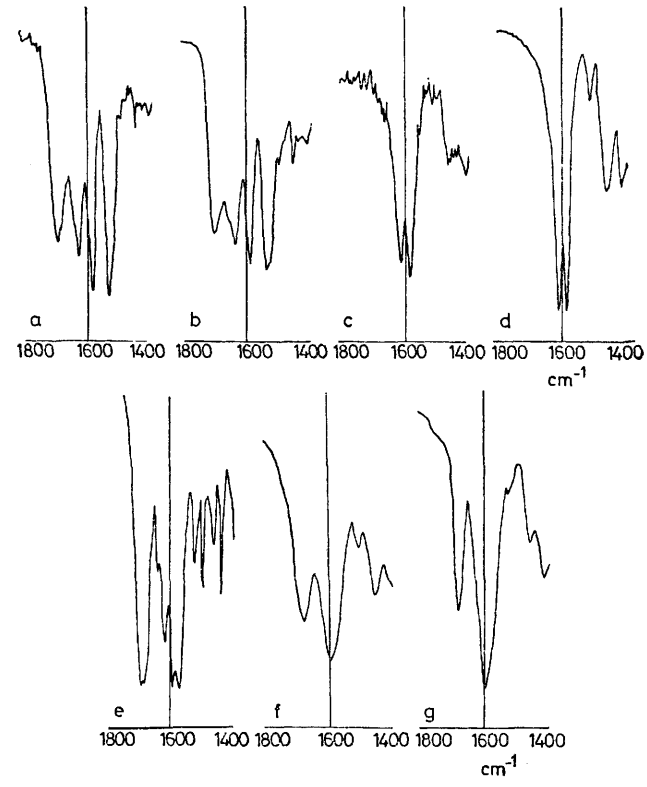


FIGURE 3. FTIR spectra of various substituted guanosines measured as micro KBr discs prepared from lyophilization or films deposited from MeOH solutions. Spectra f and g are partial spectra of compounds (**3**) and (**4**). a, 1-$PhCH_2$ (KBr); b, 1-$PhCH_2$ (MeOH); c, 6-$PhCH_2$ (KBr); d, 6-Pr^i (KBr); e, 2-Ph (KBr); f, 2-(**2**) (KBr); g, 2-(**1**) (MeOH).

§ See Ref. 8. It has also been applied to adducts derived from bases other than guanosine (A. M. Jeffrey, K. Grzeskowiak, and I. B. Weinstein, unpublished).

¶ Spectra were originally taken as solutions to minimize solid interactions.

Micro KBr pellets were made after lyophilizing an aqueous solution of the adduct and KBr; this procedure was necessary to remove the orientational effects of the sample in the solid state. The resulting spectrum (Figure 3a) of 1-benzylguanosine obtained in this manner was almost identical with a liquid film spectrum resulting from repeated application/evaporation of a MeOH solution on the KBr plate (Figure 3b), a procedure which was far more time-consuming. Dominance of the guanine unit is clear in Figures 3c and 3d where the pattern is not affected by the 6-substituent, $PhCH_2$ or Pr^i. It should be noted that the spectra are clearly different from the case of N^1-substitution. Comparisons of spectra 3e, f, and g show that although 3e shows more fine structure, all three are diagnostic for N^2-substitution and distinguishable from the two previous cases. The FTIR method again requires only a few micrograms of sample.

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