The compounds listed in Table I have previously been synthesized by alkylation of phenylacetonitrile by means of alkyl halide in the presence of sodamide.[2] The method outlined here represents an especially useful technique because of its simple manipulation, ready availability of alcohols, excellent yield and short reaction period. The full study to establish the generality and limitations of the reaction will be reported in a subsequent paper.

In a typical example, 3.5 g. (0.15 mole sodium was added portionwise to 65 g. (0.5 mole) of *n*-octyl alcohol and the mixture was heated until all the sodium came into solution. To the preheated solution (200~210°) was added dropwise a mixture of 17.6 g. (0.15 mole) of phenylacetonitrile and 18.4 g. (0.135 mole) of methyl benzoate with stirring. In less than 5 minutes distillation of methanol started briskly. After cessation of methanol distillation the whole mixture solidified to pale-yellow cake which was heated for additional 10 minutes. The cooled mixture was solved in water, the oily layer taken up in ether, the ethereal layer washed with water, dried over potassium carbonate and distilled. A fraction, b.p. 145~150°/2 mm. was collected and weighed 25.5 g. (74.1% based on phenylacetonitrile). IR ν_{max}^{liq} cm^{-1} : 2242 (C≡N). *Anal.* Calcd. for $C_{16}H_{23}N$: C, 83.78; H, 10.11; N, 6.11. Found : C, 83.81; H, 10.00; N, 6.55.

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2) A. Rossolymo : Ber., **22**, 1237 (1889).

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On the Stoichiometric Interactions between 4-Nitroquinoline 1-Oxide and Deoxyribonucleosides, with Special Reference to the Mode of Binding of the Carcinogen to DNA*[1,2]

It has been shown that 4-nitroquinoline 1-oxide(4-NQO), a potent carcinogen,[1] binds to DNA by Nagata, *et al.*[2] by means of flow dichroism method and difference spectrum method and by Malkin, *et al.*[3] by means of thin-layer chromatography. There seems, however, to be some discrepancy of opinions with regard to the nature of binding sites : while Nagata, *et al.* emphasized that 4-NQO molecules attach to the adenine and guanine residues of DNA (4-NQO being oriented parallel to the planes of the bases), Malkin, *et al.* suggested that guanine is not involved in the interaction and obtained no proof of involvement of adenine. Quite recently, the authors observed that 4-NQO produces

*1 This paper constitutes Part XIII of a series entitled "Electronic Properties of N-Heteroaromatics." Part XII : This Bulletin, **15**, 1621 (1967).

*2 Abbreviations : DNA, deoxyribonucleic acid; dAdo, deoxyadenosine; dGuo, deoxyguanosine; dThd, deoxythymidine; dCyd, deoxycytidine; 4-NQO, 4-nitroquinoline 1-oxide; 4-NPO, 4-nitropyridine 1-oxide.

1) W. Nakahara, F. Fukuoka, T. Sugimura : Gann, **48**, 129 (1957).
2) C. Nagata, M. Kodama, Y. Tagashira, A. Imamura : Biopolymers, **4**, 409 (1966).
3) M. F. Malkin, A. C. Zahalsky : Science, **154**, 1665 (1966).

hypochromic effects on the UV absorption spectra of deoxyribonucleosides as well as on the absorption spectrum of DNA,[4] which were indicative of perturbation of electronic states of DNA bases by 4-NQO. In the present communication we wish to report further informations on the nature of the 4-NQO-DNA interaction. It will be shown, on the basis of analysis of new absorption bands exhibited in the visible difference spectra of mixed systems of 4-NQO and monomeric units of DNA, that complexes of 1:1 molar ratio are formed between 4-NQO and any one of the four bases of DNA in aqueous medium. Molar extinction coefficients and formation constants of the complexes will also be given, and discussion will be made on the mode of interaction of 4-NQO with the bases as well as with DNA. These examinations have been undertaken because the decrease of absorbances in the UV region led us to expect an increase of absorbances in other spectral regions. Non-carcinogenic 4-nitropyridine 1-oxide (4-NPO)[5] has also been tested in comparison with the quinoline compound.

The sources, solvent, and dissolution procedure of DNA, deoxyribonucleosides and the N-oxide compounds were the same as described in the preceeding paper,[4] but considerably higher concentrations were required in these experiments. Spectrophotometrically pure adenine and guanine were used. The measurements of difference spectra were carried out at approx. 15° with a Shimadzu Model MPS-50 recording spectrophotometer by using cells of 1 cm. optical path.

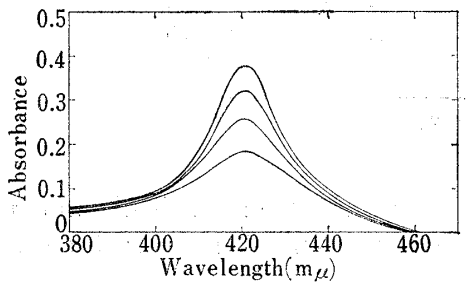

Fig. 1. Difference Spectra, dAdo plus 4-NQO *vs.* 4-NQO

Concn. of dAdo: (curves from top to bottom) $2.5\times10^{-2}M$, $2.0\times10^{-2}M$, $1.5\times10^{-2}M$, and $1.0\times10^{-2}M$.
Concn. of 4-NQO: $2.0\times10^{-3}M$.

The difference spectra of mixtures of dAdo and 4-NQO versus free 4-NQO are recorded in Fig. 1. A new absorption band was produced in the vicinity of 420 mμ, absorbance of which being increased linearly with increasing concentration of nucleoside. Similar difference spectra have been observed for the mixed systems of other nucleosides (dGuo, dThd, and dCyd) and 4-NQO (see Table I for approx. λ_{max} values). In addition, there is no doubt that base moieties of nucleosides are responsible for the production of the observed difference bands because new absorption bands were exhibited also in the mixed systems of 4-NQO and either adenine (around 420 mμ) or guanine (around 422 mμ).

In contrast to the marked effects of the quinoline compound, none of the mixed systems of deoxyribonucleoside and 4-NPO exhibited appreciable alterations in the difference spectra.

In order to have a more complete picture of the interaction, experimental data were applied to the Benesi-Hildebrand equation[6] and it was found that 1:1 complexes were formed between 4-NQO and each of the four deoxyribonucleosides. The values of molar extinction coefficient, ε, and formation constant, K, for each complex were also estimated from Benesi-Hildebrand plots, which are listed in Table I. It is noteworthy from the table that K values for the purine nucleoside systems are distinctly larger than those for the pyrimidine nucleoside systems.*3 This is in good accord with the reported fact

*3 M. Kodama, Y. Tagashira, A. Imamura, C. Nagata (Proc. Jap. Cancer Assoc., 24th Annual Meeting, 116 (1965)) suggested that absorbances of difference spectra of purine nucleoside—4-NQO systems were larger than those of pyrimidine nucleoside—4-NQO systems, but no data were given concerning the position of absorption bands nor the concentration conditions.

4) T. Okano, K. Uekama: This Bulletin, **15**, 1251 (1967).
5) H. Endo: Gann, **49**, 151 (1958).
6) H.A. Benesi, J.H. Hildebrand: J. Am. Chem. Soc., **71**, 2703 (1949).

that 4-NQO bound more strongly with apyrimidinic acid than with apurinic acid.[2] In Table I energy levels of the highest molecular orbitals, $\lambda_{h.o.}$, and values of polarizability, α, of each nucleobase are also listed. In view of the electron-donating character of nucleobases[7] and the electron-accepting character of 4-NQO,[8,9] we are led quite naturally to the presumption that the observed new absorption bands might be caused through charge-transfer interactions. And it is quite within the bounds of possibility that this is actually the case because a distinct reciprocally correspondent relationship exists between K and $\lambda_{h.o.}$ values (Table I). Moreover, the extent of magnitude of both ε and K values indicates that the difference bands most likely arise from complexes of n-π type*4 However, possibility of participation of driving forces of other types such as dispersion and polarization which. might work cooperatively with charge-transfer force, cannot be precluded because correspondent relationship is found also between K and α values (Table I), though charge-transfer force may be more important in respects of specificity[10,11] and strength of interaction.

Table I. Interactions between 4-NQO and Deoxyribonucleosides

Nucleosides	λ_{max} (mμ)	ε	K	λ h.o.[a] (β unit)	α[b] (Å^3)
dGuo	422	769	13.7	0.307	14.4
dAdo	420	800	12.8	0.486	13.9
dThd	427	200	5.56	0.510	12.0
dCyd	426	161	2.48	0.595	11.0

a) λ h.o. (see text) of the corresponding nucleobases (ref. 7).
b) Polarizabilities of the corresponding nucleobases (ref. 10).

In Fig. 2 is shown the difference spectrum of mixture of calf-thymus DNA and 4-NQO versus free 4-NQO which was measured under the same conditions as in the case of the nucleoside 4-NQO systems. The spectrum, which has wavelength maximum in the vicinity of 415 mμ, is quite similar to that presented by Nagata, *et al.*,[2] who measured the difference spectrum subsequently to the procedure of equilibrium dialysis. Although the locations of wavelength maxima of the new absorption bands produced by 4-NQO were somewhat susceptible to variation of concentration condition of the solutions concerned, noticeably the locations and shapes of the difference bands of mixed systems of monomeric units of DNA and 4-NQO are seen to be well reflected on the difference spectrum of the DNA-4-NQO system.

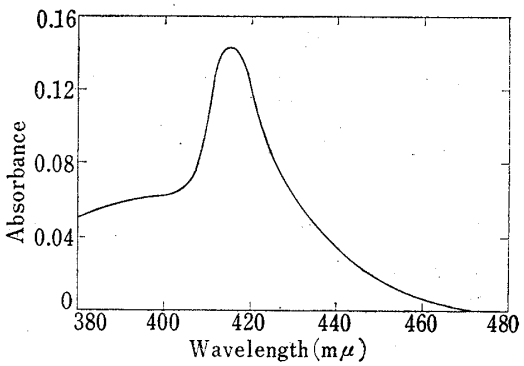

Fig. 2. Difference Spectrum, Calf-thymus DNA (0.4 mg./ml.) plus 4-NQO (2×10^{-3} M) *vs.* 4-NQO ($2\times10^{-3}M$)

Correspondingly to what was observed at the nucleoside level, 4-NPO-DNA system did not exhibit any appreciable alterations in the difference spectrum.

*4 In this connection n-donor character rather than π-donor has been exemplified by Slifkin (see ref. 11).

7) B. Pullman, A. Pullman : "Quantum Biochemistry," appendix (1963). Interscience Publishers, New York & London.
8) G. Karreman : Annals N.Y. Acad. Sci., **96**, 1029 (1962).
9) C. Nagata : Gann, **54**, 401 (1963).
10) B. Pullman, P. Claverie, J. Caillet : Science, **147**, 1305 (1965).
11) M.A. Slifkin : Biochim. Biophys. Acta, **103**, 365 (1965).

The authors have examined the UV[4] and the visible difference spectra of mixed systems of either 4-NQO or 4-NPO and each of the four deoxyribonucleosides or DNA. And it may be said from the following observations that the base moieties (adenine, guanine, thymine and cytosine residues, but the purine residues more effectively than the pyrimidine residues) do act as attachment sites for 4-NQO, although the secondary structure of DNA may have some influence on the interaction[2] : (1) changes produced in the difference spectra of mixed systems of 4-NQO and nucleosides or nucleobases in the visible as well as UV regions are well reflected on the difference spectra of 4-NQO-DNA system, (2) the order of tendencies of the monomeric units to bind with 4-NQO (purine nucleosides>pyrimidine nucleosides) is in accord with that observed at the polymer level (apyrimidinic acid>apurinic acid[2]), and (3) 4-NPO, which does not produce any difference bandat the monomer level, does not give any sign of interaction also with DNA.

Malkin, *et al.*,[3] on the basis of their finding that actinomycin D gave no evidence of intereference on the interaction of 4-NQO with DNA, suggested that the guanine residues do not act as attachment sites for 4-NQO. However, it may be pointed out in this regard that whereas actinomycin is bound to DNA through hydrogen-bonding interactions,[12] 4-NQO is most likely bound to DNA through charge-transfer interactions as described above.

The present results have shown quite definitely that while carcinogenic 4-NQO binds with the monomeric units of DNA, non-carcinogenic 4-NPO does not. It is expected accordingly that the biological activity of 4-NQO and its related compounds might be interpreted in terms of moleculer events (association of small molecules). Work along this line is being performed, and further details will be given elsewhere in the near future.

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12) L.D. Hamilton, W. Fuller, E. Reich : Nature, **198**, 538 (1963).