From the slope of this line, the approximate rate constant of the second-order reaction may be estimated. The intercept of the line might suggest the participation of the first-order reaction. A detailed account of the present paper will be published in near future.

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Received May 10, 1968

[Chem. Pharm. Bull. 16(7)1411—1413(1968)]

UDC 615.277.4.011.015.1

Effects of DNA on Free Radical Production from the Carcinogens 4-Nitro- and 4-Hydroxyamino-quinoline 1-Oxides in Aqueous Medium at 77°K[1]

Since the discovery of the potent carcinogen 4-nitroquinoline 1-oxide (4-NQO)[2] much attention has been focussed on the mode of action of this compound. Among the carcinogenic compounds structurally related to 4-NQO, 4-hydroxyaminoquinoline 1-oxide (4-HAQO) is of particular interest because it may be a proximate form of 4-NQO *in vivo*.[3] The present paper deals with the effect of deoxyribonucleic acid (DNA) on the electron spin resonance (ESR) spectroscopic behaviors of 4-NQO and 4-HAQO in an aqueous medium at 77°K, and the experimental results described below show that DNA facilitates free radical production from 4-NQO and hinders free radical production from 4-HAQO. In carrying these studies forward the authors could refer to the groundwork laid by Nagata and his co-workers, who measured the ESR spectra of 4-NQO[4] and 4-HAQO[5] in various solvents at room temperature.

Materials and methods used in the present experiments were as follows: 4-NQO,[6] mp 153—154°, and 4-HAQO hydrochloride,[7] mp 192—193° (decomp.), were synthesized in this laboratory. Calf-thymus DNA of type II preparation of Sigma Chemical Co. was used. All solutions (pH 7.0; ionic strength, $\mu=0.1$) were prepared with 0.05 M sodium phosphate buffer mixture. DNA solution was prepared according to the directions of Stone, *et al.*[8] except the phosphate buffer solution was used as a solvent. The intactness of DNA in the solution was satisfactory from the magnitude of hyperchromicity exhibited by heat denaturation. All solutions were shielded from the light before ESR measurements. ESR spectra

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5) *a*) C. Nagata, N. Kataoka, A. Imamura, Y. Kawazoe, and G. Chihara, *Gann*, **57**, 323 (1966); *b*) N. Kataoka, A. Imamura, Y. Kawazoe, G. Chihara, and C. Nagata, *Bull. Chem. Soc. Japan*, **40**, 62 (1967); *c*) N. Kataoka, S. Shibata, A. Imamura, Y. Kawazoe, G. Chihara, and C. Nagata, *Chem. Pharm. Bull.* (Tokyo), **15**, 220 (1967).
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were run on a Japan Electron Optics Laboratory JEX-3BX spectrometer with 100 kc/s magnetic filed modulation and 5 gauss modulation width. The quartz sample-tube (0.5 cm in diameter) was placed in a Dewar flask containing liquid nitrogen fitted into the sample cavity for measurements at 77°K. Photoirradiation was carried out with a 500 W superhigh-pressure mercury lamp (Ushio HMB-500 type) set at a distance of 60 cm from the center of the sample-tube in the ESR apparatus.

The ESR spectra of free radicals produced from 4-NQO and 4-HAQO in the presence or absence of DNA and/or irradiated ultraviolet light are shown in Fig. 1. Owing probably to the low-temperature ESR measurements the spectra were exhibited as single broad lines. It is evident, however, from the magnitude of g-values (g=2.006 for all the radicals observed) that these signals have arisen from organic free radicals. DNA did not give rise to any ESR signal under the present condition irrespective of photoirradiation.

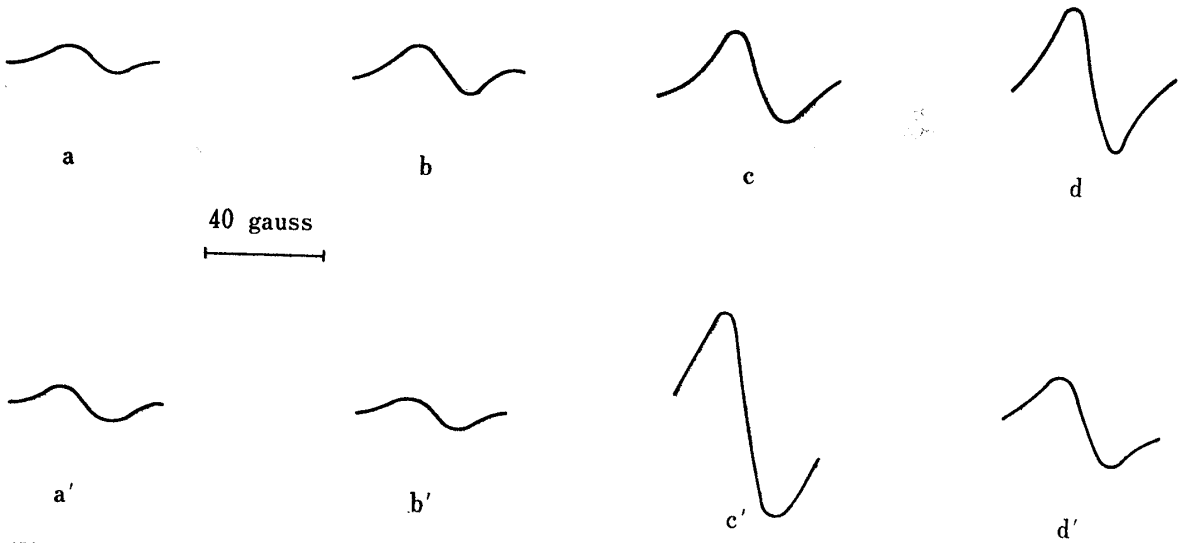

Fig. 1. Effects of DNA on the Electron Spin Resonance Spectroscopic Behaviors of 4-Nitroquinoline 1-Oxide (a—d) and 4-Hydroxyaminoquinoline 1-Oxide (a'—d') in Aqueous Medium (pH 7.0) at 77°K

a, a': without DNA, without photoirradiation b, b': with DNA, without photoirradiation
c, c': without DNA, immediately after UV-irradiation d, d': with DNA, immediately after UV-irradiation
Concn. (for both single and mixed solutions): 4-nitroquinoline 1-oxide, 2×10^{-3} M; 4-hydroxyaminoquinoline 1-oxide, 5×10^{-5} M; DNA, 0.05%

In a frozen solution at 77°K without photoirradiation, 4-NQO displayed a small signal in the spectrum (Fig. 1, a), and intensity of this signal became slightly larger when DNA was added to the same sample (Fig. 1, b). Under photoirradiation, 4-NQO displayed a radical signal of sizable intensity (Fig. 1, c), and intensity of this signal was enhanced still more by the presence of DNA (Fig. 1, d).

A weak ESR signal was displayed by 4-HAQO without photoirradiation (Fig. 1, a'); intensity of this signal became markedly larger when the sample was irradiated with ultraviolet light (Fig. 1, c'), indicating that the free radical production was accelerated by light. This is in accord with the results obtained by Nagata, *et al.*[5a] in alkaline solution at room temperature. Noticeably the observed signals are quite similar to those of the free radicals produced from the same compound in solid state.[5a] The addition of DNA caused no appreciable change in the signal intensity of the free radical produced from 4-HAQO without photoirradiation (Fig. 1, b'). Under photoirradiation, however, intensity of the large signal was decreased by the presence of DNA (compare c' and d' in Fig. 1).

The above experimental results show clearly that DNA facilitates free radical production from the carcinogen 4-NQO and hinders free radical production from the carcinogen 4-HAQO.

At present we have no positive data to go upon identification of radical structures. It is noticeable, however, that the observed effects of DNA on free radical production from 4-NQO and from 4-HAQO were in the opposite direction, one being accelarative and the other retardative. As to this, we are of the preliminary opinion that the opposite effects of DNA might be interpreted consistently in terms of charge transfer between DNA and N-oxide compounds. During the course of an investigation on the *in vitro* interaction of 4-NQO and related carcinogens with cellular materials, we have found that charge transfer occurs from the base moiety of DNA to 4-NQO[9] as well as from the former to 4-HAQO.[10] So that it may be quite possible that DNA, througn its tendency to push electrons toward N-oxide compounds, accelerated reductive radical production from 4-NQO and retarded oxidative radical production from 4-HAQO.

With a view to attaining more concrete picture of the present results, studies are being continued and further details will be published later.

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Received May 17, 1968

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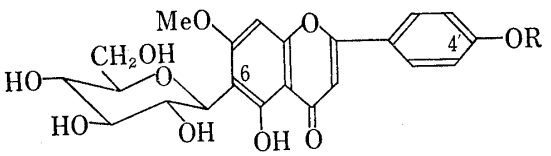
[Chem. Pharm. Bull. 16(7)1413—1415(1968)]

UDC 581.19 : 547.972.2.02

Experiments showing the Identity of Swertisin and Flavocommelitin

Flavocommelitin is an aglycone of a pale yellow flavonoid component involved in commelinin,[1] *i.e.*, a blue crystalline metallo-anthocyanin previously isolated by one of us (K.H.) from the blue *Commelina* flowers. According to K. Takeda, *et al.*,[2] the sugar-free moiety of this flavonoid was shown to be 6-C-glucopyranosylgenkwanin (I), and its glycoside, flavocommelin, is 4′-O-glucoside (II) of flavocommelitin, as shown by the following formula. In the mean time, however, it was found that the same structure had already been assigned by the two of the present authors (M.K. and T.T.) to swertisin,[3] a pale yellow substance isolated from the herb of *Swertia japonica* MAKINO by T. Nakaoki[4] in 1927.



Swertisin *viz.* flavocommelitin (I: R=H),
Falvocommelin (II: R=glucose resdiue).

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