diacetate and thioketal (XIII and XIV, as mixtures), 3β,22–diacetoxy–5α–cholestane (XVa, oily), 5α–cholestane–3β,22–diol (XVb, mp 168—170°, $[\alpha]_{578}^{16}$ +18°), 3β,22–dibenzoyloxy–5α–cholestane (XVc, mp 205—207°, $[\alpha]_{578}^{16}$ +17°). The diol (XVb) and its dibenzoate (XVc) were found to be identical with authentic samples of the correspoinding compounds derived from natural 22–hydroxycholesterol (IV) by direct comparison.

The synthesis of ecdysone from the dioxolactone (II) and 22–isoecdysone from another dioxolactone (III) have already been accomplished.[5,7] Accordingly, it is concluded that the configuration of the 22–hydroxyl group in natural 22–hydroxycholesterol (IV) is the same as that of ecdysone (I). If the configuration assgined in ecdysone is considered to be unequivocal, natural 22–hydroxycholesterol must be formulated as 5–cholestene–3β,22β_F–diol.

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Research Laboratory, Teikoku Hormone Mfg. Co., Ltd.
Shimosakunobe, Kawasaki–shi

Hiromu Mori
Kenyu Shibata
Kiyoshi Tsuneda
Masanobu Sawai

Kyoritsu Colledge of Pharmcy
Shibakoen, Minato–ku, Tokyo

Kyosuke Tsuda

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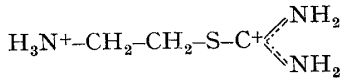
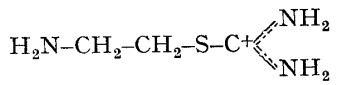
Possibility of the Second-order Reaction concerning the Transguanylation of 2-Aminoethylisothiuronium Salt

2–Aminoethylisothiuronium (AET) salt, a protective agent against a lethal dose of the ionizing radiation,[1] is transguanylated in the physiological condition to 2–mercaptoethylguanidine,[2] which may be an active form of this compound. The rate of the transguanylation is rapid, and the ionized form of AET, which is a reactive species,[3] is half transformed within one minute at 5°.[4] This paper communicated that the half life (τ) of the reaction was inversely proportional to the concentration of the ionized AET.



AET (RNH_3^+)



Ionized AET (RNH_2)

1) R. Shapira, D.G. Doherty, and W.T. Burnett, Jr., *Radiation Res.*, **7**, 22 (1957).
2) J.X. Khym, R. Shapira, and D.G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957).
3) A. Hanaki, T. Hino, S. and Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1446 (1967).
4) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), submitted.

The transguanylation was followed by the potentiometric method as described previously.[4] The half life at 5° in the presence of various amounts of alkali was presented in Table I. The value of τ appears to depend on the amount of alkali added, from which the concentration of RNH_2 can be calculated by equation (1),

$$[NaOH]=a[R]_0=[RNH_2]+[OH^-]-[H^+] \qquad (1)$$

where a, $[RNH_2]$ and $[R]_0$ represent equivalent alkali per AET, the concentrations of the ionized and total AET, respectively. The rate constant expressd by the reciprocal of τ increases with the concentration of RNH_2. If the transguanylation is the first-order reaction, τ should be constant irrespective of the amount of alkali. On the other hand, if the reaction involves the second-order reaction, τ becomes proportional to the reciprocal of the reactant concentration,.

Table I. Transguanylation of AET in the Presence of Varying Amounts of Alkali

Alkali a equiv.	$[RNH_2]$ 10^{-3}M	Half life τ (min)	Rate constant $1/\tau$ (min^{-1})
0.125	1.0	1.70	0.59
0.250	2.0	0.90	1.11
0.375	3.0	0.62	1.61
0.500	4.0	0.52	1.92
0.625	5.0	0.44	2.27

concentration : 8.00×10^{-3} M temperature : 5°

The transguanylation of AET is accompanied with the continuous drop of pH in the solution,[5] and its rate might depend on the concentration of hydroxide ion.[6] In order to confirm clearly the dependence of τ on the concentration of RNH_2, the variation in the concentration of hydroxide ion should be kept small as possible. The relation of the pH drop to the degree of the transguanylation of RNH_2 is expressed by equation (2),[7]

$$\text{Degree of Transguanylation}=1-\frac{1-a}{a}\frac{K_a}{[H^+]} \qquad (2)$$

where K_a represents the ionization constant of AET. The value of pH drop accompanying by a definite degree of the transguanylation is shown to be constant without respect to the concentration of AET. The pH change for the 50% transguanylation is calculated as approximately 0.3. Therefore, if a definite equivalent alkali is added first, where the term $(1-a)/a$ is constant, the influence of hydroxide ion may be considered almost similar throughout the fixed degree of the reaction. The half life measured in the presence of 0.5 equivalent alkali was linearly proportional to the reciprocal of the initial concentration of RNH_2 as shown in Fig. 1. This finding indicates the possibility that the transguanylation is a second-order reaction with respect to RNH_2 molecule.

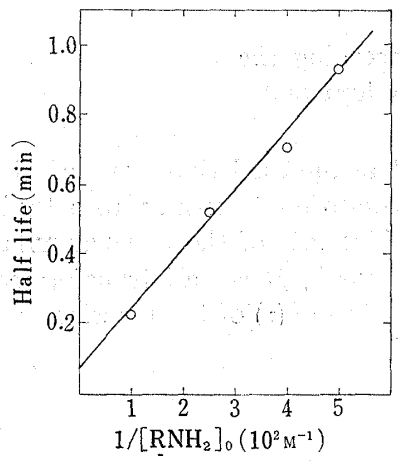


Fig. 1. Concentration Dependence of Half Life

temperature: 5°, alkali: 0.5 equivalent

5) A. Hanaki, T. Hanaki, K. Ōya, A. Andou, T. Hino, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 108 (1966).
6) A. Hanaki, P. Xumsaeng, T. Hino, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), submitted.
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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.

National Institute of Radiological Sciences
Anagawa-4, Chiba-shi

AKIRA HANAKI

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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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2) W. Nakahara, F. Fukuoka, and T. Sugimura, *Gann*, **48**, 129 (1957).
3) H. Endo and F. Kume, *Gann*, **56**, 261 (1965); T. Sugimura, K. Okabe, and H. Endo, *ibid.*, **56**, 489 (1965); Y. Kawazoe, M. Tachibana, K. Aoki, and W. Nakahara, *Biochem. Pharmacol.*, **16**, 631 (1967).
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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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