

colorless needles, m.p. 103~104° (500 mg. or 37%). *Anal.* Calcd. for  $C_{16}H_{24}O_4N_4$ : C, 57.14; H, 7.19; N, 16.66;  $C_2H_5O$ , 40.20. Found: C, 57.40; H, 7.10; N, 16.34;  $C_2H_5O$ , 40.21.

**Ethyl 3-Dimethylaminomethyl-5,7-diethoxy-1,4,6-triazaindene-2-carboxylate Methiodide (PPy-XXXVI)**—A mixture of PPy-XXXV (200 mg.; 0.6 mmole), EtOH (2 ml.), and  $CH_3I$  (100 mg.; 0.7 mmole) was kept in a closed vessel, when a small amount of colorless crystals separated out soon. After being kept standing overnight the crystals were filtered off, the filtrate was concentrated, and the remaining syrupy substance was dissolved in a little MeOH. To the MeOH solution was added a great amount of petr. ether, the separated resinous substance was scrubbed against the wall, and the solidified product was recrystallized from  $Me_2CO$ -AcOEt (1:2) into colorless needles, m.p. 180° (positive to flame reaction of I). *Anal.* Calcd. for  $C_{17}H_{27}O_4N_4I$ : C, 42.69; H, 5.69; N, 11.71. Found: C, 43.00; H, 5.91; N, 11.60.

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### Summary

1,4,6-Triazaindene and its derivatives corresponding to the naturally occurring purine bases were synthesized from the 1,4,6-triazaindenes reported in the preceding paper. The structures of these derivatives were confirmed by investigating them on their physico-chemical properties such as ultraviolet absorption and nuclear magnetic resonance spectra.

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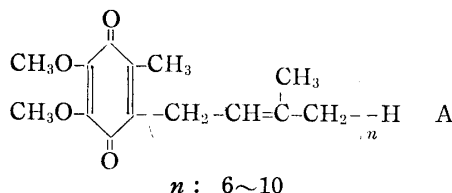
### 143. Isuke Imada, Yasushi Sanno, and Hiroshi Morimoto :

Photochemical Reaction of Ubiquinone (35). I.\*<sup>1</sup>

Photochemical Reaction of Ubiquinone (35).\*<sup>2</sup>

(Research Laboratories, Takeda Chemical Industries, Ltd.\*<sup>3</sup>)

In 1957, one of quinones was isolated from beef heart by Crane, *et al.* and made clear that the quinone was involved in the electron transport system. The quinone was named coenzyme Q by Lester, *et al.* On the other hand, a substance having an absorption peak at 272 mμ was isolated from the liver of vitamin A-deficient rats by Heaton, *et al.* and was referred to as ubiquinone by



\*<sup>1</sup> This paper constitutes Part II of a series entitled "On the Components of Yeast"; Part I: Biochem. Z., **340**, 155 (1964).

\*<sup>2</sup> A brief report of this work was published as a Communication to the Editor in this Bulletin, **11**, 815 (1963).

\*<sup>3</sup> Juso-nishino-cho, Higashiyodogawa-ku, Osaka (今田伊助, 三野 安, 森本 浩).

Morton, *et al.* Coenzyme Q was proved identical with ubiquinone by the identification of their degradation products and was evidenced to have the structure, A ( $n=10$ ).<sup>1)</sup>

As reported previously<sup>2)</sup> one (I.I.) of the authors extracted ubiquinone from the cells of various microorganisms and investigated the method of purifying it. Especially he obtained ubiquinone (35) (I) from *Candida utilis* in a good yield. Thereafter, in the course of investigating the stability of I, I was found to be changed by sunlight.<sup>3)</sup> Therefore, the products were isolated and their chemical structures, mode of their formation and their biological activities were clarified, which will be reported successively.

I in ethanol was irradiated with sunlight and the ultraviolet spectrum of the reaction mixture was investigated occasionally. And it was found that 275  $m\mu$  (quinone absorption) characteristic of ubiquinone homologues gradually decreased and the absorption around 240  $m\mu$  increased (Fig. 1). When the ultraviolet spectrum of the reaction mixture was investigated after addition of sodium borohydride, 290  $m\mu$  characteristic of the hydroquinone compounds of ubiquinone homologues was observed and the absorption around 270  $m\mu$  increased (Fig. 2). Crane<sup>1)</sup> also observed the phenomenon but did not

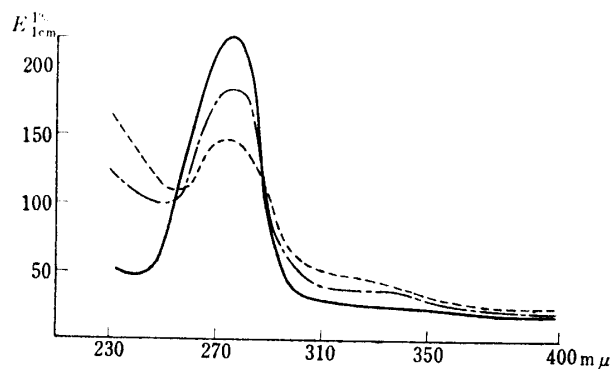


Fig. 1. Ultraviolet Spectra of Photochemical Reaction Product of Ubiquinone (35) (EtOH)

— original  
 - - - 150  
 . . . 300 ( $10^6$  UV-lux  $\times$  hr.)

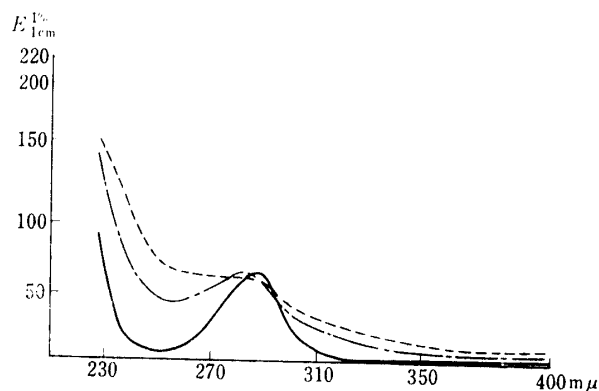


Fig. 2. Ultraviolet Spectra of Photochemical Reaction Product of Ubiquinone (35) after Addition of Sodium Borohydride (EtOH)

— original  
 - - - 150  
 . . . 300 ( $10^6$  UV-lux  $\times$  hr.)

clarified the detail. So according to the method of Lester and Ramasarma (referred to as PPC hereinafter), the authors developed the above-mentioned sample on filter paper (Whatman No. 1) treated with silicone (silicone No. 550F, Dow Corning, Co.), using propanol-water (4:1) as solvent and treated with 2% potassium permanganate to give the results shown in Fig. 3. The sample was also subjected to thin-layer chromatography by the method of Wagner, *et al.*<sup>4)</sup> Namely, the sample was developed on a thin layer of silica gel G (Merck, Co.) treated with a solution of paraffin in ether, using acetone-water (9:1) as solvent and colored with sulfuric acid (referred to as TLC hereinafter) (Fig. 4). As seen in Figs. 3 and 4, the number of colored spots increased with sunlight irradiation time, and after 3 million UV-lux  $\times$  hr. at least seven spots appeared. The reaction mixture was poured on a column packed with silicic acid-Hyflo Super-Cel (2:1),

- 1) References cited in G.E.W. Wolstenholme and C.M. O'Connor: "Ciba Foundation Symposium on Quinones in Electron Transport," (1961), J. & A. Churchill Ltd., London.
- 2) I. Imada, S. Wada, H. Shimazono, N. Miyata, M. Miwa: Nippon Nôgei-Kagaku Kaishi, **37**, 580 (1963).
- 3) H. Shimazono, M. Terao, S. Kawajiri, I. Imada, K. Aoki: Japan. Pat., under application.
- 4) H. Wagner, L. Hörhammer, B. Dengler: J. Chromatog., **7**, 211 (1962); H. Wagner, B. Dengler: Biochem. Z., **336**, 380 (1962).

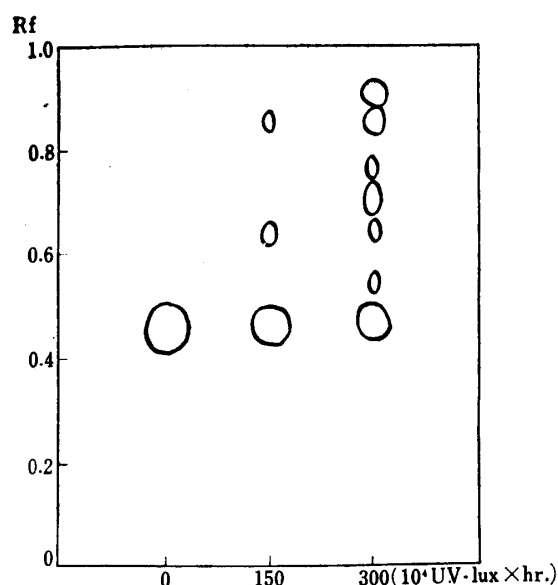


Fig. 3. Paper Partition Chromatography of Photochemical Reaction Product of Ubiquinone (35)

Solvent system:  $\text{PrOH-H}_2\text{O}$  (4:1)

Paper: Whatman No. 1 impregnated with silicone

Ascending method

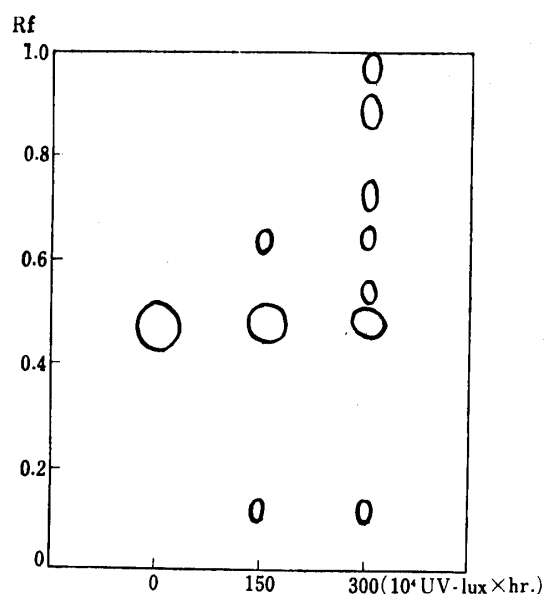


Fig. 4. Thin-Layer Chromatography of Photochemical Reaction Product of Ubiquinone (35)

Solvent system:  $\text{Me}_2\text{CO-H}_2\text{O}$  (9:1)

Thin layer: silica gel G impregnated with paraffin

when yellow, orange, violet, and brown chromatograms were obtained, which were eluted with mixtures of hexane and chloroform in various ratios to yield nine fractions (A~I). The yields and Rf-values are shown in Table I. The photochemical reaction was

TABLE I. The Rf-Value of Each Fraction separated on Sillicic Acid Column Chromatography of Reaction Mixture

Fraction name	Solvent system hexane : $\text{CHCl}_3$	Volume of solvent (ml.)	Yield (%)	Rf-values of PPC <sup>a)</sup>	
A	20 : 1	50	4	0.65	
B	20 : 1	160	41	0.47	
C	20 : 1	15	5	(0.47)	0.78
D	20 : 1	165	7	0.56	
E	20 : 1	35	3	(0.56)	0.73
F	10 : 1	150	4	0.65 (0.71)	
G	10 : 1	85	4	0.71	(0.86)
H	5 : 1	170	17		0.86
I	1 : 1	450	15		0.92

a) PPC was as indicated in Fig. 3.

Figures bracketed are Rf-values of by-products.

effected also in methanol with the same results. Some fractions affected their ultra-violet spectra when reduced with sodium borohydride. This may suggest the presence of some substances, which, like I, have a relation with oxidoreduction system in cells. In fact, some of them had a biological activity as will be described later in detail. There are some light sources, which effect photochemical reactions like sunlight. So the authors used a mercury lamp having 254  $\text{m}\mu$  to give a change to I. The reaction mixture was investigated from its ultraviolet spectrum (Fig. 5) and PPC to obtain the

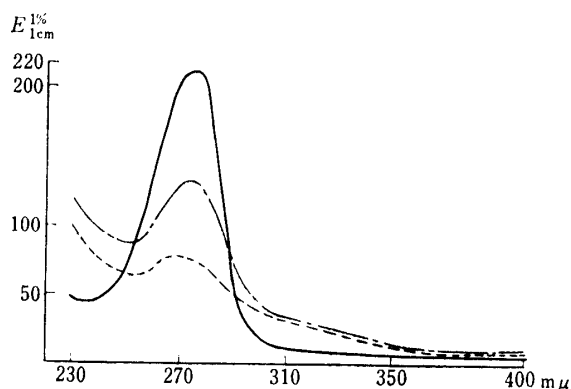


Fig. 5. Ultraviolet Spectra of Photochemical Reaction Product of Ubiquinone (35) by Mercury Lamp (EtOH)

— original  
 --- 100  
 - · - 200 ( $10^4$  UV-lux  $\times$  hr.)

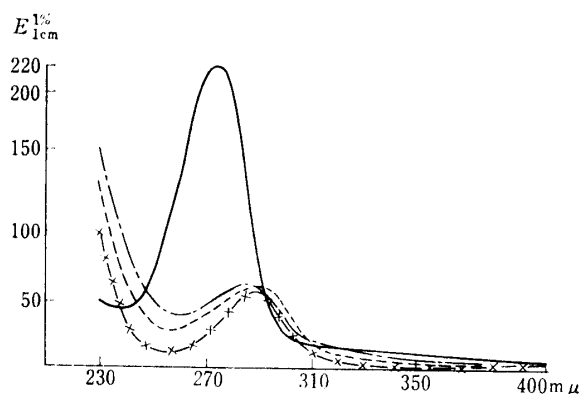
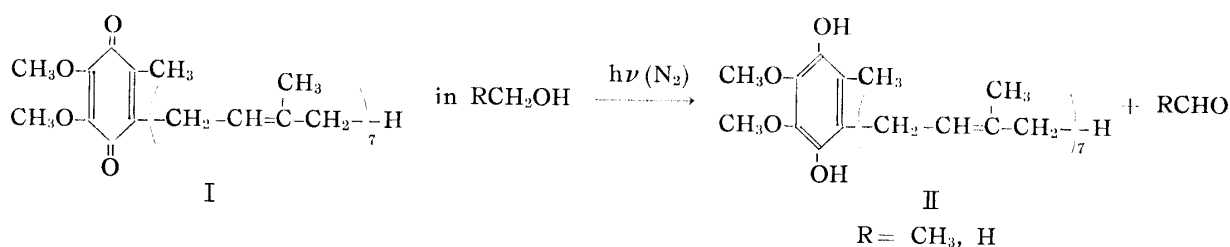


Fig. 6. Ultraviolet Spectra of Photochemical Reaction Product of Ubiquinone (35) in  $N_2$  (EtOH)

— original  
 --- 150  
 - · - 300 ( $10^4$  UV-lux  $\times$  hr.)  
 - x - x - hydroquinone compound of ubiquinone (35)

same results as the sunlight irradiation. It was reported by Gibbs<sup>5)</sup> that benzoquinone was reduced to benzohydroquinone by irradiation with sunlight in a solution of methanol and the methanol was oxidized to formaldehyde. In an attempt to observe a similar reaction with I, the compound was irradiated with sunlight in a solution of methanol to give formaldehyde which was detected by the chromotropic acid reaction.<sup>6)</sup> On the other hand acetaldehyde was detected by gas chromatography when ethanol was used as the solvent.<sup>\*4</sup> As the resulting hydroquinone (II) was readily oxidizable in the air, I was irradiated with sunlight in a sealed tube in an atmosphere of nitrogen. The color of the reaction mixture changed from orange-red to light-yellow and in course of time the absorption maximum of I at 275  $m\mu$  shifted bathochromically near to that<sup>7)</sup> of II prepared from I by Shunk's method<sup>8)</sup> (Fig. 6). From these facts, it has been found that I is converted to II accepting the hydrogen from the solvent when oxygen is absent and otherwise to other products, which will be described later in detail.



### Experimental

**Reaction of Ubiquinone (35) (I) with Sunlight** — A solution of I (300 mg.) in EtOH (50 ml.) was irradiated with sunlight (3 million UV-lux  $\times$  hr.; Matsuda's integrating type heliograph IL-1-A). The reaction mixture was evaporated to dryness under  $40^\circ$  in  $N_2$ , and the residue was dissolved in a little hexane and adsorbed on a column ( $2 \times 25$  cm.) of silicic acid-Hyflo Super-Cel (2:1) (30 g.), when yellow, orange,

\*4 Gas chromatography was carried out by Mr. T. Shima in our research laboratories.

5) H. Gibbs: Philip. J. Sci., **7**, 57 (1912); J. Plotnikow: "Allgemeine Photochemie," 734 (1936), Walter De Gruyter & Co., Berlin.

6) E. Eegriwe: Z. Anal. Chem., **110**, 22 (1937).

7) R. L. Lester, Y. Hatefi, C. Widmer, F. L. Crane: Biochim. Biophys. Acta, **33**, 169 (1959).

8) K. Folkers, C. H. Hoffman: U.S. Pat. 2,962,519.

violet, and brown chromatograms were obtained. The column was developed with the solvents shown in Table I and 5 ml. each of the eluate was investigated by PPC to give nine fractions (A~I). The yields and Rf-values in the eluate of each fraction are shown in Table I.

**Determination of the Aldehyde produced by the Photochemical Reaction of I**—i) A reaction mixture of I in MeOH was distilled on the water bath at 40° in a vessel cooled with dry ice and Me<sub>2</sub>CO, and the HCHO in the distillate was determined with chromotropic acid reaction. ii) A reaction mixture of I in EtOH was treated as above and 0.2 ml. of the distillate was adsorbed on a column (4 m. × 4 mm.) of PEG-1000·Chromosorb-W (40~60 mesh) (3:7) and acetaldehyde was determined by gas chromatography (He at 60 ml./min. at 60°).

**Irradiation of I with a Mercury Lamp**—A solution of I (119 mg.) in MeOH in a beaker was irradiated with a mercury lamp from a distance of 5 cm. (254 (main wave length), 314, 365, 405, 437, 550 mμ, 90 w., Daika-Kogyo, Co.). The light was measured with Matsuda's integrating type heliograph IL-1-A. The reaction mixture became warm to 35~40° and MeOH evaporated, which was supplemented to keep the reaction mixture at a definite volume. After the reaction, the mixture was made 25 ml. and a definite amount of the solution was diluted 200 times with EtOH and its UV-spectrum (Fig. 5) was measured. PPC of the reaction mixture was in accord with that shown in Fig. 1.

**Irradiation of I with Sunlight in Nitrogen**—I (20 mg.) was dissolved in MeOH (2 ml.) in a clear ampule, the air in the ampule was replaced by N<sub>2</sub>, and the ampule was irradiated with sunlight. The measurement of sunlight was conducted in the same manner as in the reaction in air. The reaction mixture was diluted 500 times with EtOH and its UV spectrum was measured to give the results shown in Fig. 6. PPC of the reaction mixture in N<sub>2</sub> (solvent: PrOH-H<sub>2</sub>O (7:3)) and then treatment with 2% KMnO<sub>4</sub> gave the same colored spot as the hydroquinone compound (II) of I.

### Summary

When ubiquinone (35) (I) was irradiated with sunlight in ethanol or methanol, it was converted to some substances and the absorption at 275 mμ characteristic of homologues of ubiquinone decreased, the absorption around 240 mμ increasing. The reaction product was purified by chromatography on silicic acid to give nine fractions. In this case, when ethanol was used as solvent, acetaldehyde was formed, and when methanol was used, formaldehyde was produced. The same conversion was observed by irradiation with a mercury lamp. I was converted into the hydroquinone compound (II) in a sealed tube, in which the air was replaced by nitrogen.

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