

UV $\lambda_{\text{max}}^{\text{EtOH}} \text{m}\mu (E_{\text{1cm}}^{1\%})$: oxidized form 314(105); reduced form 255(136), IR $\nu_{\text{max}}^{\text{liquid}} \text{cm}^{-1}$: 3500(OH); others are about the same as in II. NMR (Table I).

Summary

It was clarified that one of the photochemical reaction products of ubiquinone (35) (I) had the structure (II) where one of the double bonds of the isoprene side chain of I was conjugated with the quinone nucleus. It was named isoubiquinone (35). γ -Hydroxyisoubiquinone (35) (VII) was obtained by treating ubichromenol (35) (IV) with lithium aluminum hydride.

(Received May 4, 1964)

[Chem. Pharm. Bull.
12 (9) 1056 ~ 1059]

UDC 547.567 : 541.14

146. Isuke Imada, Yasushi Sanno, and Hiroshi Morimoto :
Photochemical Reaction of Ubiquinone (35). IV.*¹
Formation of Demethylubiquinone (35).*²

(Research Laboratories, Takeda Chemical Industries, Ltd.*³)

When the fraction H described in the previous report,¹⁾ which was obtained by photochemical reaction of ubiquinone (35) (I), was subjected to chromatography on silicic acid, it was adsorbed as a violet band. The eluate of the violet band was yellow probably because it contained a substance (II) which forms a violet complex salt with the magnesium contained in the adsorbent. In order to purify the eluate it was adsorbed on a column of Florisil and the column, after washing with hexane-ethanol (1:1), was eluted with methanol to give a violet eluate. As the eluted substance may be combined with magnesium in a complex combination, it was dissolved in hexane, acidified with sulfuric acid, and treated as will be described in the experimental part, giving II (m.p. 38°, Rf 0.86*⁴) as orange-red plates from methanol. From its analytical value and molecular weight II was found to have a molecular formula of $C_{43}H_{64}O_4$. It had one methoxyl and one less carbon and two less hydrogen than I ($C_{44}H_{66}O_4$), and its infrared spectrum (Fig. 1) showed newly 3350 cm^{-1} (OH) beside 1665, 1610

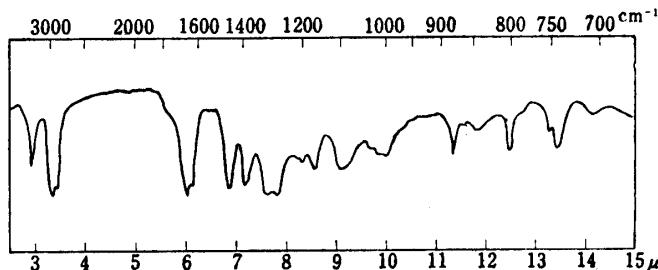


Fig. 1. Infrared Spectrum of Demethylubiquinone (35) (Liquid)

*¹ This paper constitutes Part V of a series entitled "On the Components of Yeast"; Part IV : This Bulletin, 12, 1051 (1964).

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

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

*⁴ Paper partition chromatography was carried out according to the previous report.¹⁾

1) I. Imada, Y. Sanno, H. Morimoto : This Bulletin, 12, 1042 (1964).

cm^{-1} (quinone nucleous). The ultraviolet spectrum of II has a same maximum at $275 \text{ m}\mu$ as I and the spectrum in the visible region of II exhibited a maximum at $440 \text{ m}\mu$ different from that ($405 \text{ m}\mu$) of I, and II was positive to leucomethylene blue reaction²⁾ and the product of its reduction with sodium borohydride was positive to the neotetrazolium chloride reaction.³⁾ From these facts it is evident that II has a quinone structure. That II has hydroxyl was confirmed by the fact that it gave $\text{FeCl}_3 \cdot \text{K}_3\text{Fe}(\text{CN})_6$ reaction,⁴⁾ and infrared spectrum of its acetyl derivative (III) obtained with acetic anhydride and pyridine showed no 3350 cm^{-1} (OH), which was observed in II, and newly exhibited $1790, 1190 \text{ cm}^{-1}$ (COCH_3). It is generally known that oxyanthraquinones having a hydroxyl at the carbon adjacent to the carbonyl on the quinone nucleus produce violet complex salt with magnesium contained in Florisil or slightly in silicic acid.⁵⁾ When magnesium acetate was added to a solution of II in methanol, the reaction mixture turned violet and 275 and $440 \text{ m}\mu$ in II shifted to 285 and $550 \text{ m}\mu$, respectively. From the fact the authors thought that II was produced from I by conversion of one of the two methoxyls in I to a hydroxyl, and named it demethylubiquinone (35). Reduction of II in ethanol with sodium borohydride produced a maximum in ultraviolet spectrum at $350 \text{ m}\mu$, which, however, shifted to $295 \text{ m}\mu$ after about 30 minutes (Table I). It is obvious from many

TABLE I. Reduction Time and Ultraviolet Absorption of Demethylubiquinone (35)

Time after addition of NaBH_4 (min.)	$E_{1\text{cm}}^{0.072\%}$		Time after addition of NaBH_4 (min.)	$E_{1\text{cm}}^{0.072\%}$	
	$350 \text{ m}\mu$	$295 \text{ m}\mu$		$350 \text{ m}\mu$	$295 \text{ m}\mu$
0	0.360	0.182	15	0.200	0.240
1	0.370	0.105	25	0.150	0.270
5	0.295	0.140	30	0.160	0.310
10	0.235	0.180			

examples that $295 \text{ m}\mu$ is the maximum shown by the phenyl nucleus of the hydroquinone compound, but it is not clear what reduction intermediate produced $350 \text{ m}\mu$. The fact that the proton number of the methoxyl of II is $1/2$ of that of I judging from relative band area of its nuclear magnetic resonance spectrum (Table II), and I and II are the

TABLE II. Nuclear Magnetic Resonance Spectra

Proton type	Demethylubiquinone (35)		Ubiquinone (35)		τ -Value ^{a)}
	No. of protons Calcd.	No. of protons based on 1 OCH_3	No. of protons Calcd.	No. of protons based on 2 OCH_3	
=CH (chain)	7	7	7	7	4.9~5.0
$-\text{OCH}_3$	3	(3)	6	(6)	6.0
=C-CH ₂ - (nucleus)	2	2	2	2	6.7~6.9
=C-CH ₃ (nucleus)	} 27	30	27	24	7.9~8.0
-CH ₂ -C= (chain)					
=C-CH ₃ (chain)	24	25	24	23	8.3~8.4

a) The bands refer to 60 Mc. spectra in carbon tetrachloride, using tetramethylsilane as an internal standards (Varian-A-60).

- 2) B. O. Linn, A. C. Page, Jr., E. L. Wong, P. H. Gale, C. H. Shunk, K. Folkers : J. Am. Chem. Soc., 81, 4007 (1959).
- 3) R. L. Lester, T. Ramasarma : J. Biol. Chem., 234, 672 (1959).
- 4) G. M. Barton, R. S. Evans, J. A. F. Gardner : Nature, 170, 249 (1952).
- 5) S. Shibata : Yakugaku Zasshi, 61, 320 (1941).

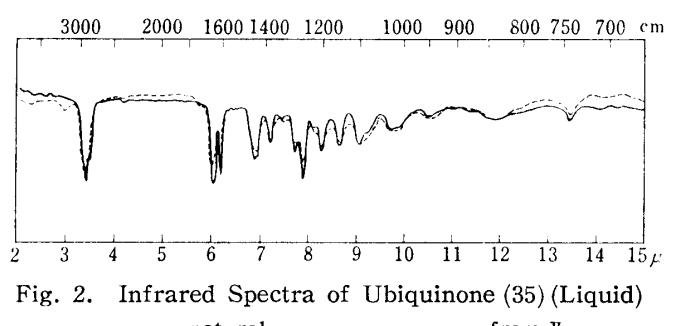


Fig. 2. Infrared Spectra of Ubiquinone (35) (Liquid)

— natural —— from II

magnetic resonance spectrum, Rf-value and analytical data was produced from I by conversion of either of the two methoxyls of I into a hydroxyl, the authors conducted the following experiment. Namely, if II could be converted to demethylubichromenol (35) (IV) by the method⁶⁾ which converted I to ubichromenol (35), the position of the hydroxyl in question would be clarified by investigating the relation between the two hydroxyls. For the purpose, II was heated with triethylamine at 100° in nitrogen in a sealed tube, when the reaction progressed as expected, and the ultraviolet spectrum of the resulting IV exhibited 277, 285, 312 m μ , showing that it has chromenol structure. As is obvious from the route of II \rightarrow IV, the relation between the two hydroxyls is ortho or meta. Formerly, Brauer⁷⁾ investigated the color reactions of *o*-, *m*-, and *p*-dihydroxybenzene with phosphomolybdic acid and found that they gave different color reactions. IV exhibited a blue color reaction, showing its two hydroxyls to be in *o*-relation and, therefore, the structure of II was decided as demethylubiquinone (35).

Recently Glover, *et al.*⁸⁾ found rhodoquinone, a substance having a structure similar to that of I, in a kind of photosynthetic bacterium, *Rhodospirillum rubrum*. It is interesting to know that all the things mentioned above are concerned with photochemical reaction. The biological activities of II will be reported separately.

same in the isoprene side chain, supports the above assumption strongly. Next, to revert II to I it was methylated with dimethyl sulfate and anhydrous potassium carbonate in dry acetone and purified by chromatography on Florisil, and it was found that the resulting methyl derivative of II was the same with I in ultraviolet, infrared (Fig. 2) and nuclear

magnetic resonance data. To evidence that II was

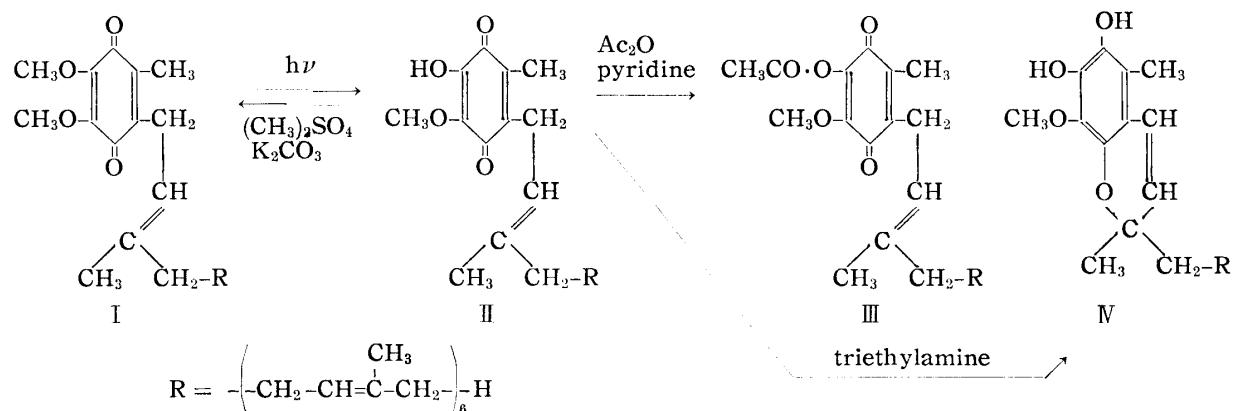
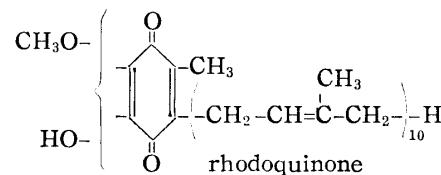
demethylubichromenol (35) (IV) by the method⁶⁾ which converted I to ubichromenol (35), the

position of the hydroxyl in question would be clarified by investigating the relation

between the two hydroxyls. For the purpose, II was heated with triethylamine at 100°

in nitrogen in a sealed tube, when the reaction progressed as expected, and the ultra-

visible spectrum of the resulting IV exhibited 277, 285, 312 m μ , showing that it has chromenol structure. As is obvious from the route of II \rightarrow IV, the relation between the two hydroxyls is ortho or meta. Formerly, Brauer⁷⁾ investigated the color reactions of *o*-, *m*-, and *p*-dihydroxybenzene with phosphomolybdic acid and found that they gave different color reactions. IV exhibited a blue color reaction, showing its two hydroxyls to be in *o*-relation and, therefore, the structure of II was decided as demethylubiquinone (35).



Experimental^{*5}

Formation of Demethylubiquinone (35) (II) by Photochemical Reaction—A solution in hexane (1 ml.)

*5 Melting point is uncorrected.

6) I. Imada, H. Morimoto: This Bulletin, 12, 1047 (1964).

7) K. Brauer: Chem. Ztg., 50, 553 (1926).

8) J. Glover, D. R. Threlfall: Biochem. J., 85, 14p (1962).

of the fraction H (48 mg.) obtained previously¹⁾ was adsorbed on a column (1×5 cm.) of Florisil (5 g.). The column was washed with hexane-EtOH (1:1), eluted with MeOH, and the eluate was evaporated to dryness in N_2 *in vacuo*. A solution in hexane (2 ml.) of the violet residue (36 mg.) was acidified by addition of MeOH (0.1 ml.) and 3*N*H₂SO₄ (0.1 ml.), when the violet color turned to yellow. The hexane solution was washed with H₂O, dried on Na₂SO₄ and evaporated to dryness in N_2 . The residue was dissolved in MeOH and the solution was left standing in a cool place to separate orange-red plates (15 mg.), m.p. 38°. *Anal.* Calcd. for C₄₃H₆₄O₄: C, 80.12; H, 9.95; 1OMe, 4.81; mol. wt., 644. Found: C, 79.71; H, 10.02; OMe (Zeisel), 4.75; mol. wt. (Barger), 626±25.

UV $\lambda_{\text{max}}^{\text{EtOH}} \text{m}\mu (E_{\text{1cm}}^{1\%})$: oxidized form 275 (133); reduced form 350 (51)→295 (37). IR $\nu_{\text{max}}^{\text{liquid cm}^{-1}}$: 3350 (OH); 1665, 1610 (quinone); 1258, 1207, 1150, 1093 (OMe). NMR (Table II).

Acetylation of II: Formation of the Acetate of II—To a solution of II (24 mg.) in Et₂O (0.4 ml.) were added Ac₂O (0.2 ml.) and pyridine (0.2 ml.) and the mixture was left standing at room temperature overnight. The solvent was evaporated *in vacuo*, the residue was dissolved in Et₂O (5 ml.), and the solution was washed with 3*N*H₂SO₄, 2% NaHCO₃ and H₂O, dried over Na₂SO₄ and evaporated to dryness. The oily residue was crystallized from a small amount of MeOH to give III as yellow crystals. IR $\nu_{\text{max}}^{\text{liquid cm}^{-1}}$: 1790, 1190 (COCH₃).

Methylation of II: Formation of Ubiquinone (35) (I)—A solution of II (125 mg.) in Me₂CO (5 ml.) was refluxed for 30 min. with K₂CO₃ (0.5 g.) and (CH₃)₂SO₄ (0.5 ml.), when the color of the reaction mixture changed from orange→deep violet→yellow. The solvent was evaporated and the residue was dissolved in hexane (2 ml.) and adsorbed on a column packed with Florisil (5 g.). The column was eluted with hexane-CHCl₃ (1:1) and the oily substance obtained by evaporating the eluate was dissolved in a little EtOH and kept in a cool place with MeOH to separate yellow plates. *Anal.* Calcd. for C₄₄H₆₆O₄: 2OMe, 9.42. Found: 8.97. UV $\lambda_{\text{max}}^{\text{EtOH}} \text{m}\mu$: oxidized form 274; reduced form 290. IR (Fig. 2).

Ring-closure of II with Triethylamine: Formation of Demethylubichromenol (35) (IV)—II (5 mg.) and triethylamine (0.1 ml.) were heated at 100° in N_2 in a sealed tube for 15 min., when the color of the reaction mixture changed from violet to yellow. The reaction mixture was evaporated to dryness at 40°, the residue was dissolved in benzene, and the solvent was distilled azeotropically *in vacuo* to remove the amine. The residue, which showed a single colored spot in thin-layer chromatography,*⁶ gave blue-color reaction with 1% phosphomolybdic acid. $\lambda_{\text{max}}^{\text{EtOH}} \text{m}\mu$: 277, 285, 312.

The authors wish to express their deep gratitude to Dr. K. Tanaka for his kind encouragement and to Mr. N. Matsumoto for his technical assistance throughout this series of investigation (Part II~Part V). The authors are also indebted to the members in charge of elementary analyses and measurement of physical constants.

Summary

It was made clear that one of the photochemical reaction products of ubiquinone (35) (I) had the structure (II) in which one of the two methoxyls of I was converted to a hydroxyl. And it was named demethylubiquinone (35). II was converted to demethylubichromenol (35) (IV) by treating with triethylamine, and the position of the hydroxyls of II was decided from the fact that the two hydroxyls of IV gave a color reaction with phosphomolybdic acid, showing that they are in ortho-relation.

(Received May 4, 1964)

*⁶ Developed on silica gel G (E. Merck A.G.) with CHCl₃, and a spot was obtained with H₂SO₄.