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144. Isuke Imada and Hiroshi Morimoto: Photochemical Reaction of Ubiquinone (35). II.*1 Formation of Ubichromenol (35).*2

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Ubichromenol[1] is the general name of the compounds produced by a ring formation between the quinone nucleus and one of the double bonds of side chain in ubiquinone (UQ).*4 As in UQ, some homologues of ubichromenol with different isoprene side chains have been reported but it was doubted whether they are present in nature, and their physiological significance has yet not been made clear. They were named according to the carbon number of the isoprene side chain of the UQ to which they correspond in the structure (for example, ubichromenol (35) : UC (35)).

Formation of Ubichromenol (35) by Photochemical Reaction

The fraction A with Rf 0.65 described in the previous report[2] contained impurities beside the main component (II), so it was purified by chromatography on Florisil into a pale yellow oil. From its elementary analysis it was found to have the same molecular formula, $C_{44}H_{66}O_4$, as UQ (35) (I), and in its infrared spectrum (Fig. 1) absorptions at 1640 and 1610 cm^{-1} characteristic of the quinone nucleus disappeared, and instead absorptions at 3560 (OH), 3050, 2950, 1605, 1490 cm^{-1} (aromatic nucleus) were newly observed. As it gave phenolic color reactions with $FeCl_3$, $FeCl_3 \cdot K_3Fe(CN)_6$,[3] and infrared spectrum of its acetyl derivative obtained with acetic anhydride and pyridine showed no 3560 cm^{-1} (OH), which was observed in II and newly exhibited 1770, 1210 cm^{-1} ($COCH_3$), it seems to have a phenol structure. It was concluded that from ultraviolet spectra of II and its reduction product (III), one of the double bonds conjugates with the phenol nucleus. That is, ultraviolet spectrum of II showed absorption maxima at 275, 282, and 332 mμ, and it was more bathochromic than that of III. It is well known that in general when the phenyl structure is conjugated with a double bond, its absorption maximum makes some bathochromic shift and this is observed in the relation between benzene compounds and styrene compounds. Absorption at 332 mμ of II is the bathochromic effect caused by conjugation of B-band (292.5 mμ) of III with a double bond, and from the fact that III is no longer bathochromic, the above assumption is right. The assumption was also supported by nuclear magnetic resonance spectra (Table I). Of 3.58 and 4.53 τ (doublet, J=10 c.p.s.) (one of double bonds in

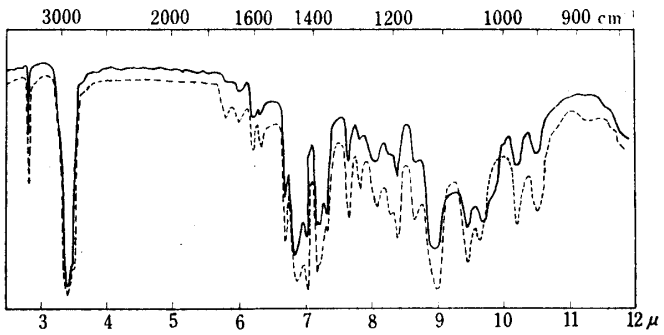

Fig. 1. Infrared Spectra of Ubichromenol (35) (CCl_4)
——— with light
– – – – – with triethylamine

*1 This paper constitutes Part III of a series entitled "On the Components of Yeast"; Part II : This Bulletin, **12**, 1042 (1964).

*2 A brief report of this work was published as a Communication to the Editor in this Bulletin, **12**, 739 (1964).

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*4 Ubiquinone is referred to UQ in this report.

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, Y. Sanno, H. Morimoto : This Bulletin, **12**, 1042 (1964).
3) G. M. Barton, R. S. Evans, J. A. F. Gardner : Nature, **170**, 249 (1952).

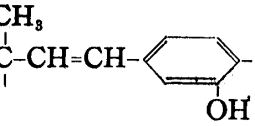
the side chain) the former is a proton adjacent to the phenyl nucleus and the latter is smaller than τ-value of the general vinyl proton by 0.5 p.p.m. As 8.65 τ of a methyl protons is also smaller than that of the general methyl protons by 0.5 p.p.m., a partial structure of  was concluded. Since 4.70 τ of II was established to be proton of the hydroxyl group by the exchange with deuterium oxide and as the other protons of side chain is about the same as that of I, II was concluded to be ubichromenol (35) produced by a ring-closure between one of double bonds of the isoprene side chain and the quinone nucleus of I. Although the structure of II has as asymmetric carbon, it is an optically inactive *dl*-compound, and it was also found by the authors that I is not affected by chromatography on Florisil.

TABLE I. Nuclear Magnetic Resonance Spectra

Proton type	τ-value[a]	No. of protons based on $2CH_3O$/mole	
		Ubiquinone (35) (I)	Ubichromenol (35) (II)
=C-CH-	3.50, 3.65	—	1
=CH-C-O-C=	4.45, 4.60	—	1
-OH	4.70	—	1
=CH- (chain)	4.92	—	6
	4.90~5.00	7	—
$-OCH_3$	6.10, 6.20	—	6
	6.10	6	—
$=C-CH_2-C=$	6.7~6.9	2	—
$=C-CH_3$ (nucleus)	7.88	—	3
	7.9~8.00	3	—
$=C-CH_2-$	8.00	24	24
$=C-CH_3$ (chain)	8.40	24	21
$-O-C-CH_3$	8.65	—	3

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

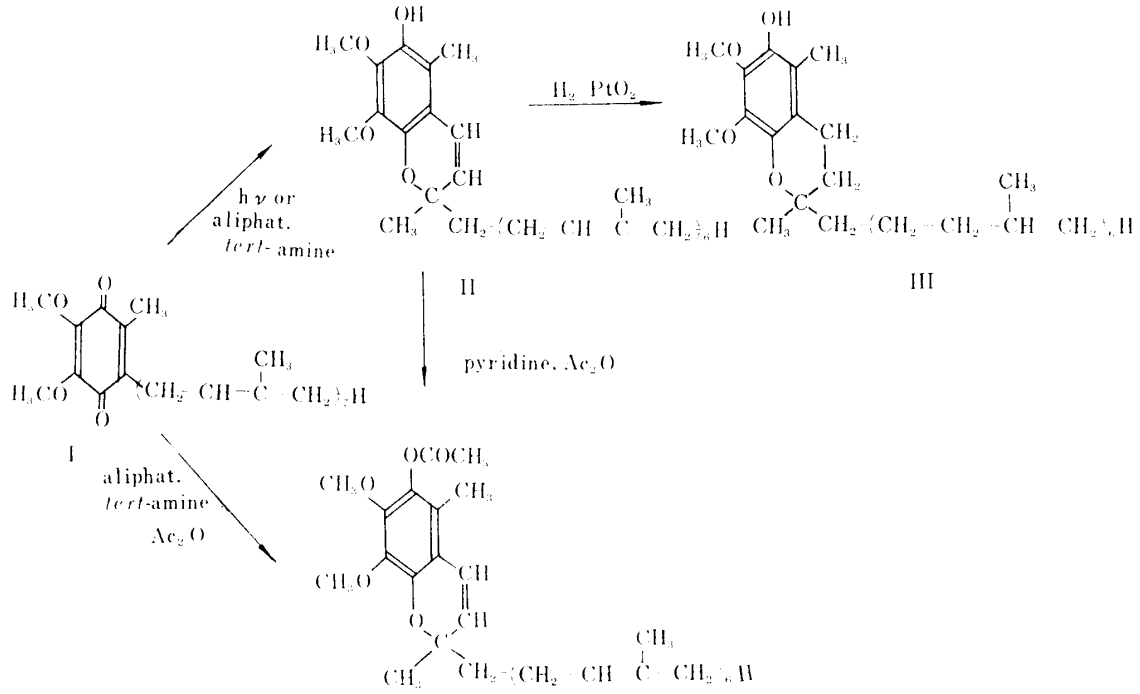
Synthesis of Ubicromenol (35) from Ubiquinone (35)

It is known that UQ is converted to UC by treating with basic alumina,[4] pyridine[5] or sodium hydride,[6] and Linn, *et al.* made an assumption on the mechanism of this conversion. According to the assumption the reaction is effected by the catalytic action of the base. UQ is rather unstable against alkali[7] and its methoxyl group is exchanged with an ethoxyl group by treating with sodium ethoxide,[8] for example. It is also easily thinkable from examples[9] of alkoxy derivatives of benzoquinone that UQ is substituted with an amino group by treating with a primary or secondary amine. With these facts in mind the authors tried to obtain II from I with a good yield by treating with an aliphatic tertiary amine, and the reaction was conducted in an atmosphere of nitrogen because the formed II is easily oxidizable by air. Triethylamine, 1-ethylpiperidine and

4) C.H. Shunk, F.R. Koniuszy, E.L. Wong, N.R. Trenner, B.H. Arison, K. Folkers: Biochem. Biophys. Research. Commun., 3, 228 (1960); J. Links: Biochim. Biophys. Acta, 38, 193 (1960); F.W. Hemming, R.A. Morton, J.F. Pennock: Biochem. J., 80, 445 (1961); B.C. Johnson, Q. Crider, C.H. Shunk, B.O. Linn, E.L. Wong, K. Folkers: Biochem. Biophys. Research. Commun., 5, 309 (1961).
5) D. McHale, J. Green: Chem. & Ind. (London), 1962, 1867.
6) B.O. Linn, C.H. Shunk, E.L. Wong, K. Folkers: J. Am. Chem. Soc., 85, 239 (1963).
7) H. Shimazono, M. Terao, S. Kawajiri, I. Imada, K. Aoki: Japan. Pat., under application.
8) B.O. Linn, N.R. Trenner, C.H. Shunk, K. Folkers: J. Am. Chem. Soc., 81, 1263 (1959).
9) W.K. Anslow, H. Raistrick: J. Chem. Soc., 1939, 1446; W. Gauss: Chem. Ber., 91, 2216 (1958).

1-methylpiperidine were used as aliphatic tertiary amine and the yields were compared. In general, when aliphatic tertiary amines are used, the reaction is completed in a short time and the yield of II is good and, therefore, its isolation is easy. When I was heated with the same amount of an amine in a sealed tube at 100°, II was produced with 80% yield and the product was identified with the same substance obtained by photochemical reaction by ultraviolet, infrared (Fig. 1), nuclear magnetic resonance spectrum and paper partition chromatography.*5

As derivatives of II, perhydroubichromanol (35) (III) was obtained by catalytic reduction of II on platinum oxide and acetylubichromenol (35) (IV) by acetylation of II with acetic anhydride. IV was also obtained directly by treating I with triethylamine and acetic anhydride. In nuclear magnetic resonance spectrum of III, 3.58, 4.53, and 4.92 τ (-CH=) disappeared and 7.48 τ (=C-CH_2-C-) appeared, and in that of IV 4.70 τ (OH) disappeared and 7.73 τ ($COCH_3$) was observed, supporting their structures, respectively.



Experimental

Formation of Ubichromenol (35) (II) by Photochemical Reaction——In the same manner as reported in the previous paper[2] except the sunlight irradiation (3 million UV-lux × hr.; Matsuda's integrating type heliograph 1 L-1-A). I (10 g.) was treated to give the fraction A (771 mg.). Unreacted I (4.47 g.) was recovered in this case. A solution of the fraction A in hexane (5 ml.) was adsorbed on Florisil (100~200 mesh, Floridin Co.) (50 g.) packed in a column (3 × 17.5 cm.) and eluted with hexane-$CHCl_3$ (8:2) (flow rate, 50ml./hr.). And the fraction (450 ml.) of the eluate containing II (investigated by PPC) was concentrated in N_2 at 40° under reduced pressure to yield II as a pale yellow oil. *Anal.* Calcd. for $C_{44}H_{66}O_4$: C, 80.19; H, 10.10. Found: C, 80.30; H, 10.03. UV λ_{max}^{EtOH} mμ ($E_{1cm.}^{1\%}$): 332 (49), 282 (121), 275 (126), 230 (shoulder). IR ν_{max}^{liquid} cm^{-1}: 3050, 2950, 1605, 1490 (aromatic ring); 3560 (OH); 1280, 1110 (OMe); 1385, 1370, 1155 (side chain). NMR (Table I). Optically inactive.

*5 Paper partition chromatography (PPC) was carried out according to the previous report.[2]

Synthesis of II from Ubiquinone (35) (I)——i) I (100 mg.) was heated with an amine (0.1 ml.) in N_2 in a sealed tube or refluxed with the amine (5 ml.) in N_2. The reaction mixture was worked up by the method to be mentioned later to determine I and II. The results are shown in Table II.

Table II. Synthesis of II by Various Amines

Condition	Time (hr.)	Temp. (°C)	Amine	II (%)	I (%)
Reflux	1/6	90	Triethylamine	73	27
	1/6	115	Pyridine	33	67
Heat in tube	2	100	Triethylamine	90	10
	2	100	Pyridine	44	56
	2	100	1-Ethylpiperidine	81	2
	2	100	1-Methylpiperidine	84	—
	2	130	1-Ethylpiperidine	96	—
	96	20	Triethylamine	46	21
	96	20	Pyridine	21	79

ii) I (1 g.) and triethylamine (1 ml.) were heated in N_2 in a sealed tube at 100° and the reaction mixture was worked up as in (i) to give the results shown in Table III.

Table III. Synthesis of II by Triethylamine

Time (hr.)	II (%)	I (%)	Time (hr.)	II (%)	I (%)
1	87	6	3	85	3
2	90	3	6	76	4

iii) I (250 mg.) was dissolved in triethylamine (1 ml.) and 0.5 ml. of the solution was diluted with multiple triethylamine to make solutions in which the ratios between I and triethylamine were 1:4, 1:8, 1:16, 1:32, and 1:64. Each of the solutions was heated in N_2 in a sealed tube at 100° for 2 hr., the reaction mixture was worked up as in (i), and I and II were determined. In each case, the yield of II was 84~95% and unchanged I was 10.0~5.4%.

iv) I (1 g.) and triethylamine (1 ml.) were heated in N_2 in a sealed tube at 100° for 1 hr., the reaction mixture was evaporated to dryness at 40° *in vacuo* and benzene was added to the residue and distilled azeotropically to remove the amine. The residue was dissolved in hexane (5 ml.), the solution was poured on a column of Florisil (50 g.) and eluted with hexane-$CHCl_3$ (8:2) (flow rate, 50 ml./hr.). The yield was 800 mg. (80%).

Determination of I and II in the Reaction Mixture——The reaction mixture was evaporated to dryness at 40° *in vacuo* and benzene was added to the residue and distilled azeotropically to remove the amine. The residue was dissolved in hexane to make a solution containing 100~50 mg. of the reactant, poured on a column (1×8 cm.) packed with Florisil (3 g.) and eluted with hexane-$CHCl_3$ (7:3) (flow rate 60 ml./hr.). The eluate (180 ml.) was evaporated to dryness *in vacuo*, the residue was dissolved in EtOH, and I in the solution was determined from difference between the oxidized and reduced forms in absorption coefficients as 275 mμ by the method of Crane, *et al.*[10] II was determined from the absorption coefficient at 332 mμ of the reduced from.

Catalytic Reduction of II: Formation of Perhydroubichromanol (III)——A solution of II (506 mg.) in EtOH-AcOH-cyclohexane (1:1:1) (50 ml.) was shaken in H_2 in the presence of PtO_2 (100 mg.), when 123 ml. of H_2 was absorbed in 2 hr. (theoretic. 120 ml.). To the reaction mixture was added H_2O (20 ml.) and extracted with three 50 ml.-portions of petr. ether. The extract was washed with H_2O, dried and evaporated to dryness *in vacuo*. The residue was dissolved in hexane (1 ml.), poured on a column (1.5×25 cm.) packed with Florisil (25 g.) and eluted with hexane-$CHCl_3$ (1:1). The fraction (150 ml.) of the eluate corresponding to III (investigated by PPC) was evaporated to dryness, when III was obtained as a pale yellow oil (409 mg., 79%). *Anal.* Calcd. for $C_{44}H_{80}O_4$: C, 78.51; H, 11.98. Found: C, 78.85; H, 11.85. UV λ_{max}^{EtOH} mμ ($E_{1cm}^{1\%}$): 292.5 (54.4).

Formation of Acetylubichromenol (35) (IV)——i) To a solution of II (50 mg.) in pyridine (5 ml.) was added Ac_2O (0.05 ml.) and the mixture was left standing at room temperature for 5 hr. The reaction

10) F. L. Crane, R. L. Lester, C. Widmer, Y. Hatefi: Biochim. Biophys. Acta, 32, 73 (1959).

mixture was poured into a large amount of ice water and extracted with three 10 ml.-portions of Et_2O, and the Et_2O solution was evaporated at 40° *in vacuo* to dryness to yield IV as a pale yellow oil.

ii) A mixture of I (506 mg.), triethylamine (1 ml.) and Ac_2O (0.2 ml.) was heated in N_2 in a sealed tube at 100° for 1 hr. and the reaction mixture was poured into a large amount of ice water and extracted with hexane. The extracted substance (542 mg.) was dissolved in a small amount of hexane and adsorbed on Florisil (27 g.) packed in a column (1.5 × 25 cm.). The column was washed with hexane (400 ml.) (flow rate, 25 ml./min.) and hexane-$CHCl_3$ (9:1) (1260 ml.) to remove impurities and then eluted with hexane-$CHCl_3$ (4:1) (600 ml.). The eluate was evaporated to dryness *in vacuo*, when IV was obtained as a pale yellow oil (449 mg., 84%). *Anal.* Calcd. for $C_{46}H_{68}O_5$: C, 78.81; H, 9.78. Found: C, 78.65; H, 9.82. UV λ_{max}^{EtOH} mμ ($E_{1cm}^{1\%}$): 274.5 (115.5), 283 (104.5), 315 (35.8). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770, 1210 ($COCH_3$).

Summary

It was made clear that ubichromenol (35) (II) was one of the products of photochemical reaction of ubiquinone (35) (I). It was also found that I was converted to II in good yield with an aliphatic tertiary amine. Perhydroubichromanol (35) (III) and acetylubichromenol (35) (IV) were obtained as derivatives of II.

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145. Isuke Imada and Hiroshi Morimoto: Photochemical Reaction of Ubiquinone (35). III.*1 Formation of Isoubiquinone (35).*2

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The fraction D which was produced by photochemical reaction of ubiquinone (35) (I) as described in the previous report[1] contained a small amount of impurities, so it was purified by a column chromatography on Florisil and obtained as a red oil (II) showing Rf 0.56*4 by elution with hexane–chloroform (1:1). From analytical values the substance was found to have molecular formula of $C_{44}H_{66}O_4$ and its infrared spectrum (Fig. 1) was in accord with that of I except 1590 cm^{-1}. Namely, 1645, 1605 (quinone nucleus) and 1270, 1105 (OCH_3), 1385, 1380, 1155 cm^{-1} (isoprene side chain) were observed but no absorptions of hydroxyl and independent carbonyl

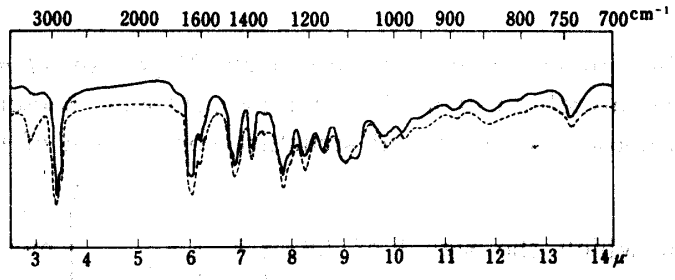

Fig. 1. Infrared Spectra of Isoubiquinone (35) (II) and γ-Hydroxyisoubiquinone (35) (VII) (liquid)
——— II ----- VII

*1 This paper constitutes Part IV of a series entitled "On the Components of Yeast"; Part III: This Bulletin, 12, 1047 (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, 森本 浩).

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

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