

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 N 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 160° 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 \times 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 N was obtained as a pale yellow oil (449 mg., 84%). *Anal.* Calcd. for $\text{C}_{46}\text{H}_{68}\text{O}_6$: C, 78.81; H, 9.78. Found: C, 78.65; H, 9.82. UV $\lambda_{\text{max}}^{\text{EtOH}}$ $\text{m}\mu$ ($E_{1\text{cm}}^{\text{EtOH}}$): 274.5 (115.5), 283 (104.5), 315 (35.8). IR $\nu_{\text{max}}^{\text{CCl}_4}$ 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 acetyl-ubichromenol (35) (N) 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 report¹⁾ contained a small amount of impurities, so it was purified by a column chromatography on Florisil and obtained as a red oil (II) showing R_f 0.56*⁴ by elution with hexane-chloroform (1:1). From analytical values the substance was found to have molecular formula of $\text{C}_{46}\text{H}_{68}\text{O}_4$ 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

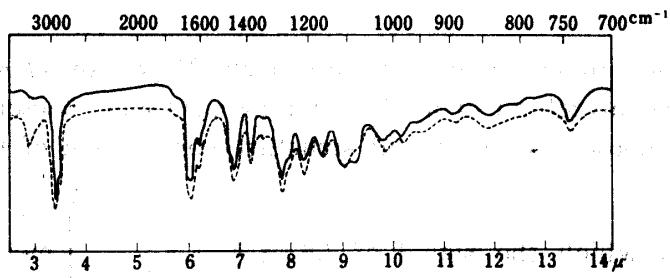


Fig. 1. Infrared Spectra of Isoubiquinone (35) (II) and γ -Hydroxyisoubiquinone (35) (VI) (liquid)

*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).

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*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).

group were found. That the substance (II) is a quinone compound was confirmed by the fact that it is positive to the leucomethylene blue reaction²⁾ and it is reduced with sodium borohydride to a compound positive to the neotetrazolium chloride reaction.³⁾ The fact seems to show that II is a quinone compound similar to I only different

in the isoprene side chain. Therefore, II was catalytically reduced in the presence of platinum oxide, the resulting hydroquinone compound was oxidized with ferric chloride to the perhydroquinone compound (III), and the product was compared with the perhydroubiquinone (35) prepared from I in the same manner, finding that they are in accord with each other in Rf-value and infrared (Fig. 2), ultraviolet, and nuclear magnetic resonance spectrum. Accordingly it

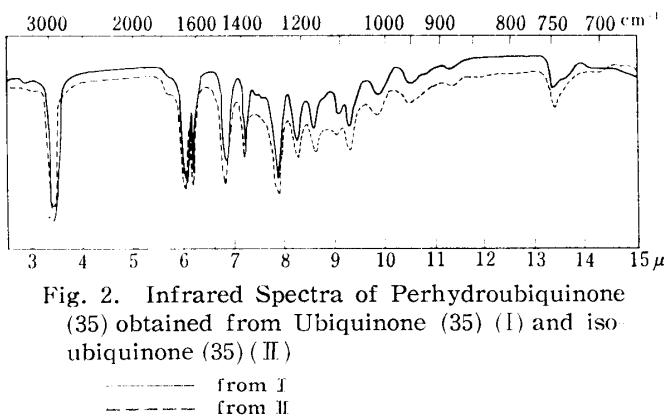
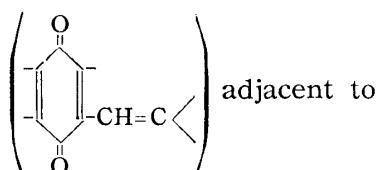


Fig. 2. Infrared Spectra of Perhydroubiquinone (35) obtained from Ubiquinone (35) (I) and iso-ubiquinone (35) (II)

— from I
- - - from II

was concluded that II is different from I in the position of the double bonds of their isoprene side chains. The ultraviolet spectrum of II has a maximum at $314 \text{ m}\mu$, which is $40 \text{ m}\mu$ bathochromic compared with that of I. In general, conjugation of dienone with double bond causes bathochromic effect, and this is observed, for example,⁴⁾ in the fact that androsta-1,4,6-triene-3,17-dione (A) which is a triene of the steroid series is $54 \text{ m}\mu$ more bathochromic than androsta-1,4-diene-3,17-dione (B). Fieser, *et al.*⁵⁾ presented an equation thereon and calculated that conjugation of a double bond results in $36 \text{ m}\mu$ bathochromic effect. As reported above, a double bond was observed at 1590 cm^{-1} in the infrared spectrum of II, which was not found in I, and the fact shows that II can have a structure where the quinone nucleus conjugates with a double bond in the isoprene side chain of I. Comparison of nuclear magnetic resonance spectrum of I with that of II (Table I) indicated that 6.8τ observed in I disappeared and newly signals were appeared at 3.78 and $8.7\sim8.8\tau$, of which 3.78τ is a vinyl proton



adjacent to the quinone nucleus. Formerly Frydman, *et al.*⁶⁾ isolated chlorobiumquinone (C) from the mycelium of a kind of photosynthetic bacterium, *Chlorobium thiosulfatophilum*, and proposed as its partial structure conjugation of the quinone nucleus with a double bond,

in which the signal of 3.8τ (shown in a similar conjugated structure) means the same as in II. And multiplet at $8.7\sim8.8\tau$ are considered as $=\text{C}-\text{C}-\text{CH}_2-$, $=\text{C}-\text{C}-\text{CH}_3$ produced by conjugation of the quinone

- 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) C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufmann, J. Pataki : J. Am. Chem. Soc., **72**, 4531 (1950); *Idem* : *Ibid.*, **72**, 4534 (1950).
- 5) L. F. Fieser, M. Fieser : "Steroids," 19 (1959), Reinhold Publishing Corporation, New York.
- 6) B. Frydman, H. Rapoport : J. Am. Chem. Soc., **85**, 823 (1963).

TABLE I. Nuclear Magnetic Resonance Spectra

Proton type	τ -value ^{a)}		
	Isoubiquinone (35) (II)	γ -Hydroxyiso- ubiquinone (35) (VII)	Chlorobium- quinone (C)
=C-CH=	3.78	3.60	3.8 (b)
-CH= (chain)	5.00 (b)	4.95 (b)	4.9 (b)
-OCH ₃	6.10 (s)	6.10 (s)	—
=C-CH ₃ (nucleus)	7.98 (s)	7.93 (s)	7.9
=C-CH ₂ -	8.00	8.00	8.0
=C-CH-	8.00	8.00	—
=C-CH ₃ (chain)	8.40	8.42	8.3~8.4
-O-C-CH ₃	—	8.70 (s)	—
=C-C-CH ₂	} 8.70~8.80	—	—
=C-C-CH ₃		—	—

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

nucleus with a double bond. As mentioned above, II is a structural isomer of I, and the authors named it isoubiquinon (35).

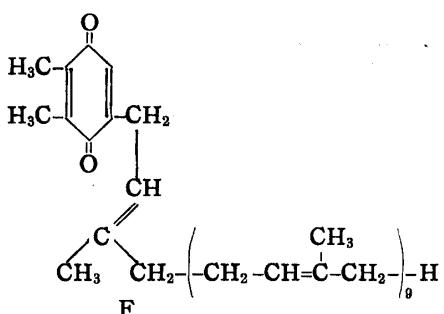
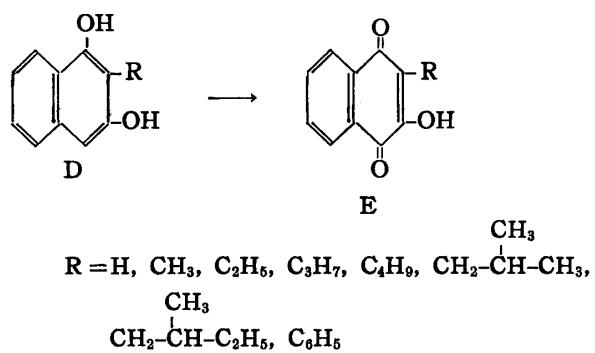
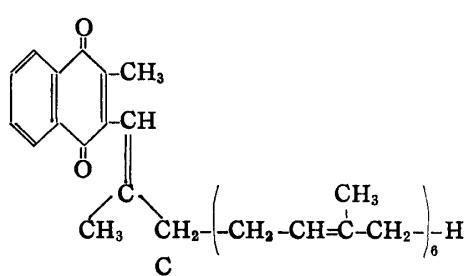
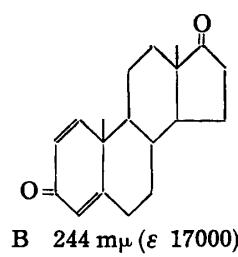
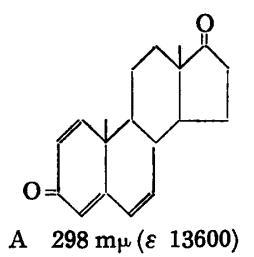
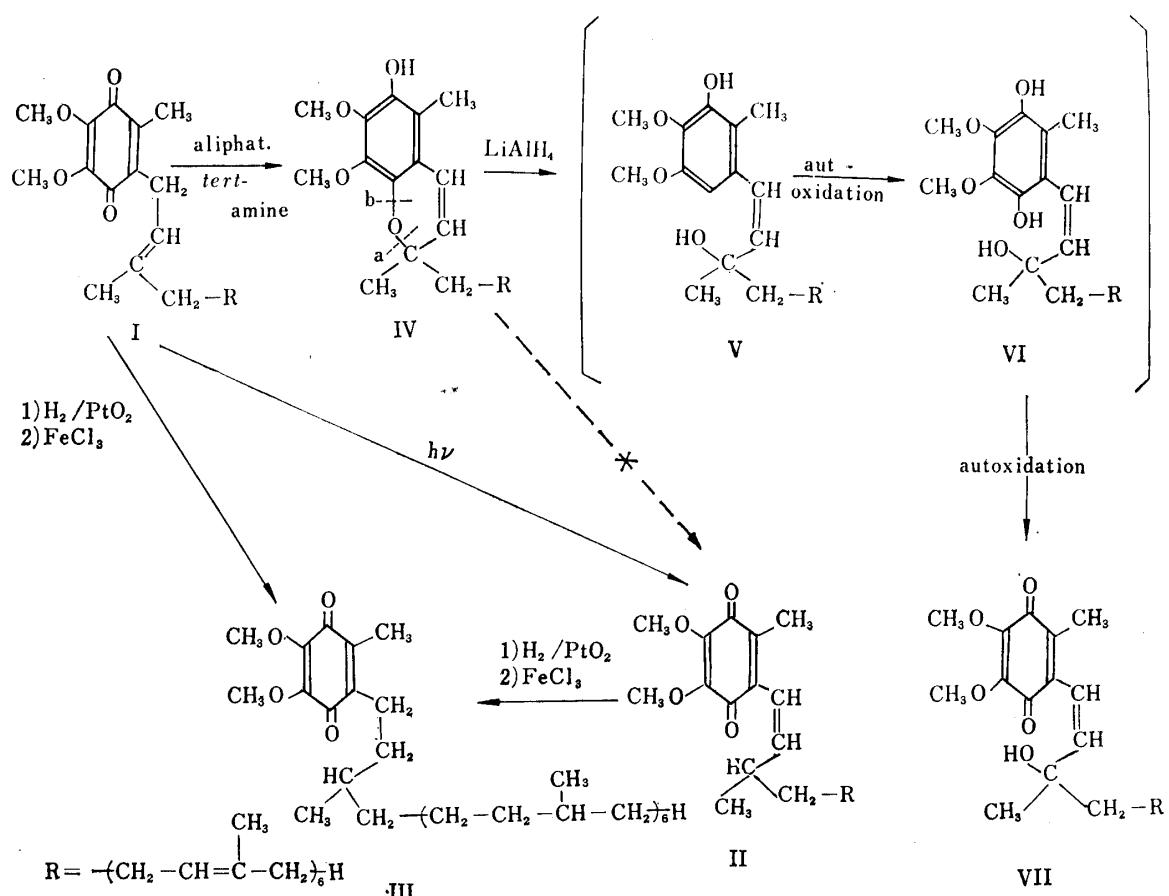
Mention was made on ubichromenol (35) (IV) in the preceding report.⁷⁾ If the pyran ring of IV could be reductively cleaved at "a," a compound ought to be formed which is represented by II except the steric configuration of the conjugated double bond. According to the method by which Schmid, *et al.*⁸⁾ reductively cleaved the ether ring of thebaine by treatment with lithium aluminum hydride, the authors, with the purpose of leading IV to II, treated II with lithium aluminum hydride in tetrahydrofuran and followed the reaction by PPC and thin-layer chromatography*. The reaction mixture was allowed to stand at room temperature overnight, and no change was observed, but after reflux for 48 hours, two new spots appeared. Neither of them were in accord with II but one of them (Rf 0.97) (VII), being positive to hydroquinone reaction with neotetrazolium chloride after reduction with sodium borohydride and to the color reaction with leucomethylene blue, is certainly a quinone compound. The reaction mixture, therefore, was treated as usual and subjected to chromatography on silicic acid, and the fraction containing VII which was eluted after the unreacted IV was further purified by chromatography on Florisil. And the purified VII was obtained as a red oil from the fraction eluted with hexane-ethanol (9:1). As was supposed from its Rf-value, VII is more hydrophilic than I (Rf 0.47) and has one more oxygen than I as its analytical value, C₄₄H₆₆O₆, shows. Although its infrared spectrum (Fig. 1) exhibits 3500 cm⁻¹ (OH), it is not substituted on the quinone nucleus because VII was negative to the FeCl₃-K₃Fe(CN)₆ reaction. As the ultraviolet spectrum of VII has a maximum at 314 m μ , it has a double bond conjugated with the quinone nucleus. From the facts mentioned above VII was considered to have the structure of isoubiquinone given to II plus one more hydroxyl group. The position of the hydroxyl group was decided by nuclear magnetic resonance spectrum

(Table I). Namely, VII has the partial structure of $\text{C}=\text{C}-\overset{\text{CH}_3}{\underset{\text{OH}}{\text{C}}}-$, because it showed the signal of one methyl group attached to the carbon adjacent to a double bond, other

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

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

8) H. Schmid, P. Karrer: Helv. Chim. Acta, 33, 863 (1950).



signals being about the same as in II and VII and therefore VII was concluded to be γ -hydroxyisoubiquinone (35). The fact that not II but VII was produced by reduction with lithium aluminum hydride suggests that not "a" hydrogenolysis was took place as expected but "b" hydrogenolysis occurred, producing a phenol compound (V) as an intermediate, and the autoxidation of V to the hydroquinone compound (VI) in the course of its purification seems to be due to the alkaline pH of the medium. An example of such an oxidation is seen in the report of Saliman, *et al.*⁹⁾ For example, 1,3-dihydroxynaphthalene (D) is oxidized to 3-hydroxy-1,4-naphthoquinone (E) with a good yield by air in a slightly alkaline medium. The authors presume that an aromatic carbon with oxygen substituents both at *o*- and *p*-position like this is smoothly oxidizable. The mechanism of the conversion of V to VII is clearly explainable from the structure of VII and gives strong support to the presumed structure of II.

Recently Chmielewska¹⁰⁾ investigated the mechanism with which plastoquinone (F) takes part in the photophosphorylation and considered as an intermediate the structure where one of double bonds of the isoprene side chain in plastoquinone is conjugated with the quinone nucleus. It is very interesting that three compounds of similar structures, isoubiquinone (35) (II), chlorobiumquinone (C) and Chmielewska's intermediate were all formed by the photoreaction. The biological activities of II and VII will be reported separately.

Experimental

Formation of Isoubiquinone (35) (II)—The fraction D (1.053 g.) was obtained by treating I (10 g.) in the same manner as described in the previous report.¹⁾ It was dissolved in hexane (5 ml.), the solution was adsorbed on Florisil (30 g.) packed in a column (2.5 \times 20 cm.) and the orange-colored band was eluted first with hexane-CHCl₃ (8:2) (500 ml.) and then with hexane-CHCl₃ (1:1) (500 ml.). The portion of the eluate showing Rf 0.56 by PPC was evaporated in N₂ at 40° *in vacuo* to give II (214 mg.) as a red oil. *Anal.* Calcd. for C₄₄H₆₆O₄: C, 80.19; H, 10.10. Found: C, 79.82; H, 9.92.

UV $\lambda_{\text{max}}^{\text{EtOH}} \text{ m}\mu (E_{\text{1cm}}^{1\%})$: oxidized form 314 (110); reduced form 255 (150). IR $\nu_{\text{max}}^{\text{liquid}} \text{ cm}^{-1}$: 1590 (>C=C< conjugated with the quinone nucleus); others are about the same as in I. NMR (Table I). Optically inactive.

Formation of Perhydroubiquinone (35) (III)—i) A solution of I (82 mg.) in EtOH-AcOH-cyclohexane (1:1:1) (50 ml.) was reduced catalytically in the presence of PtO₂ (100 mg.), when H₂ (25.4 ml.) was absorbed in 70 min. (theoretic. 22.4 ml.). The reaction mixture was extracted 3 times with petr. ether (30 ml. each) and the extract was washed with H₂O, dried and evaporated to dryness *in vacuo*. The residue was oxidized by standing overnight with an EtOH solution of FeCl₃ (0.2%, 100 ml.). The reaction mixture was concentrated to about 1/4 volume, diluted with H₂O (10 ml.), extracted with three 10 ml.-portions of petr. ether and the extract was evaporated to dryness *in vacuo*. The residue was dissolved in hexane (0.5 ml.), absorbed on Florisil (5 g.) packed in a column (1 \times 13 cm.), developed with hexane, hexane-CHCl₃ (1:1) and hexane-EtOH (1:1) successively, and the eluate with hexane-CHCl₃ (1:1) was evaporated to dryness *in vacuo* to give III as a red oil (46 mg., 56%). *Anal.* Calcd. for C₄₄H₆₆O₄: C, 78.51; H, 11.98. Found: C, 78.57; H, 12.21. UV $\lambda_{\text{max}}^{\text{EtOH}} \text{ m}\mu (E_{\text{1cm}}^{1\%})$: 279 (173).

ii) II (25 mg.) was worked up as in (i) to obtain III. The yield was 15 mg.

Reduction of Ubichromenol (35) (IV) with Lithium Aluminum Hydride: Formation of γ -Hydroxyisoubiquinone (35) (VII)—II (111 mg.) was refluxed with LiAlH₄ (0.5 g) in THF (30 ml.) for 48 hr. and to the reaction mixture was added MeOH to decompose the unreacted LiAlH₄. The resulting LiOH was filtered off and the filtrate was diluted with H₂O and extracted with Et₂O. The Et₂O solution was evaporated, the residue was dissolved in a small amount of hexane, the solution was poured on a column (2 \times 10 cm.) of silicic acid-Hyflo Super-Cel (2:1) (10 g.) and the orange-colored absorption band was eluted with hexane-CHCl₃ (1:1). The eluate was evaporated in N₂ at 40° to yield VII as a red oil (60 mg.). As the product still contained a small amount of impurities, it was dissolved in a little hexane and poured on a column (1 \times 8 cm.) of Florisil (3 g.), and the column was washed with hexane-CHCl₃ (1:1) (50 ml.) and eluted with hexane-EtOH (9:1) (50 ml.) to give VII (35 mg., 31%). *Anal.* Calcd. for C₄₄H₆₆O₅: C, 78.29; H, 9.86. Found: C, 78.12; H, 9.84.

9) G. Saliman, A. Latif: J. Chem. Soc., 1944, 55.

10) I. Chmielewska: Biochim. Biophys. Acta, 39, 170 (1960).

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.

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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

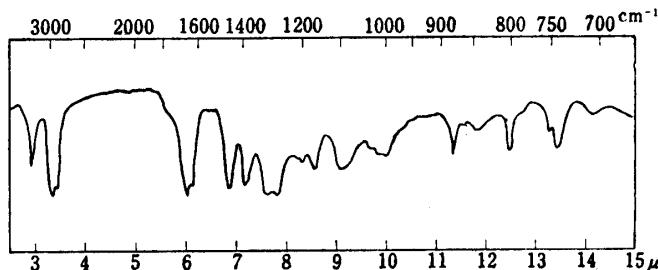


Fig. 1. Infrared Spectrum of Demethylubiquinone (35) (Liquid)

*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).