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18. Isuke Imada and Hiroshi Morimoto : Photochemical Reaction of Ubiquinone (35). V.*1 Synthesis of 2-Hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone.

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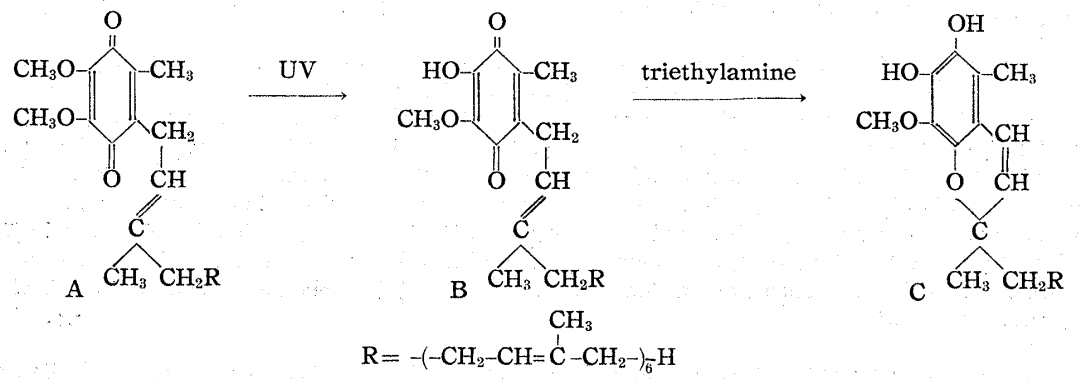
In a previous paper the authors reported that ubiquinone (35) (A) was changed by light to several compounds including demethylubiquinone (35) (B) in which one of the two methoxyls of (A) was converted to a hydroxyl, and that from the color reaction with phosphomolybdic acid of the chromenol compound (C) derived from (B), the two hydroxyls of (C) were clarified to be in *o*-relation, establishing the structure of (B).[1]



The evidence was based on the difference in the color reaction with phosphomolybdic acid between *o*- and *m*-dihydroxybenzene, and this can be further confirmed by investigating the color reaction of the chromenol compound where the two hydroxyls are in *m*-relation. For the purpose the authors intended to synthesize 2-hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone (Ⅺ). Ⅺ is easily to effect ring closure to produce the desired chromenol compound according to the finding described in Report Ⅱ,[2] and for the synthesis of Ⅺ 2-acetoxy-3-methoxy-5-methyl-*p*-benzoquinone (Ⅳ) was an important intermediate, which was prepared by the two methods (A and B) to be mentioned below.

Synthesis of 2-Acetoxy-3-methoxy-5-methyl-*p*-benzoquinone

According to the method of Pschorr, *et al.*[3] vanillin was converted to 2-nitro-3-methoxy-4-acetoxybenzaldehyde (Ⅱ) through 3-methoxy-4-acetoxybenzaldehyde (Ⅰ). With the purpose of reducing the aldehyde group of Ⅱ to alcohol (D) and further to methyl by catalytic reduction, Ⅱ was reduced with aluminum isopropoxide in isopropanol according to the method of Meerwein-Ponndorf, when exchange of the acetyl occurred unexpectedly and the product was only 2-nitro-3-methoxy-4-isopropoxybenzyl alcohol (Ⅲ), giving no desired compound. So, Ⅱ was subjected to catalytic reduction over palladium-carbon, when the hydrogen necessary for reduction of the nitro-group was consumed rapidly at room temperature and atmospheric pressure, but hydrogen required for -CHO → -CH_3 was absorbed very gradually. Investigation of the reaction mixture by thin-

*1 This paper constitutes Part Ⅵ of a series entitled "On the Components of Yeast"; Part Ⅴ: This Blletin, **12**, 1056 (1964).

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1) I. Imada, Y. Sanno, H. Morimoto: This Bulletin, **12**, 1056 (1964).
2) I. Imada, H. Morimoto: This Bulletin, **12**, 1047 (1964).
3) R. Pschorr, C. Sumuleanu: Ber., **32**, 3405 (1899).

layer chromatography found several colored spots with concentrated sulfuric acid, and as three of them were positive to diazo-reaction they may be the desired 2-amino-3-methoxy-4-acetoxytoluene, the not fully reduced aminoaldehyde and the aminoalcohol. Prolongation of reduction time and elevation of reaction temperature or pressure, however, did not raise the yield of the desired product. The reduction mixture was oxidized as such with sulfuric acid and chromic acid and Ⅳ was obtained from Ⅱ in 5.7% yield. As the yield of Ⅳ was thus poor in Method-A, an improved method was searched for. Nitration in glacial acetic acid of Ⅴ which was obtained by the hydrolysis of Ⅱ yielded 3-methoxy-4-hydroxy-2,5-dinitrobenzaldehyde (Ⅵ) in 64.4% yield, and reaction of the product in ethylmercaptan with zinc chloride and sodium sulfate in the cold gave 3-methoxy-4-hydroxy-2,5-dinitrobenzaldehyde diethylthioacetal (Ⅶ) in a good yield. When Ⅶ was acetylated, the resulting Ⅷ was reduced to the diamine compound by stirring with Raney nickel at room temperature for 10 days, and the product, without being isolated, was oxidized with ferric chloride in the usual method and purified by chromatography on Florisil, Ⅳ was obtained from Ⅷ in 3.7% yield. Thus, the method

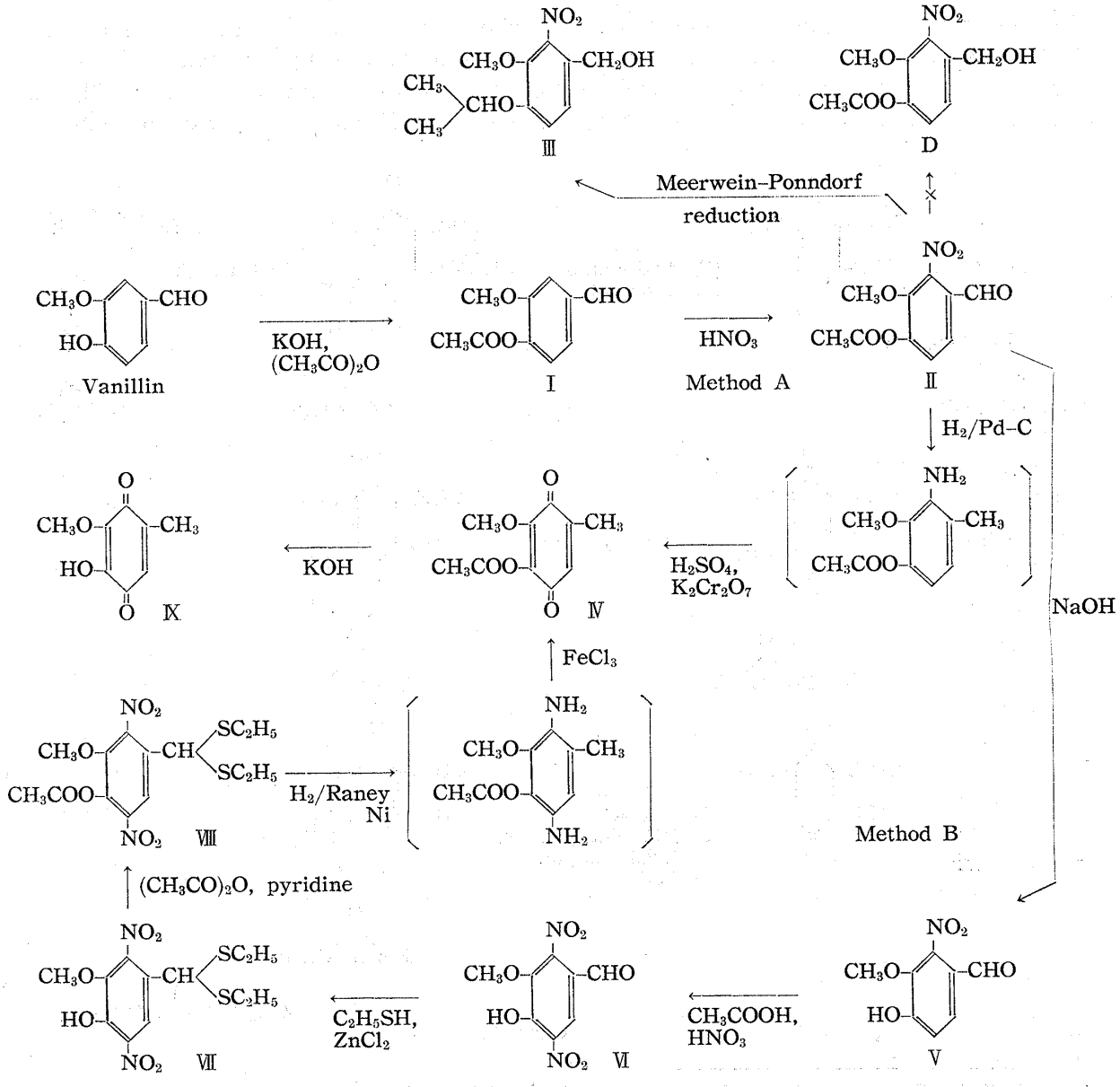

Chart 1.

(Method-B) is tedious compared with Method-A and the yield of Ⅳ was not satisfactory in this method, too. Ⅳ occurred as yellow needles melting at 48° and its ultraviolet spectrum showed absorption at 265, 358 mμ (quinone) and its infrared spectrum at 1780, 1195 (CH_3CO) and 1665, 1620 cm^{-1} (quinone).

Synthesis of 2-Hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone

Ⅳ was reduced with sodium hydrosulphite to 2-acetoxy-3-methoxy-5-methylhydroquinone (X) in ether-ethanol (1:1), and X was condensed with phytol in dioxane, using zinc chloride and boron trifluoride-diethylether according to the method of the synthesis of vitamin $K_{2(45)}$ by Noll, *et al.*[4] The product, without being purified, was dissolved in ethanol and oxidized with ferric chloride to the quinone compound and the reaction mixture was investigated by thin-layer chromatography (Table I). The samples used for comparison were Ⅳ, ubiquinone (35) (A), (B)-acetate, 5-methyl-2,3-dimethoxy-*p*-benzoquinone (E) synthesized by the known method,[5] and 5-methyl-6-phytyl-2,3-dimethoxy-*p*-benzoquinone (XIII) converted from (E). As is evident from Table I, Rf-values of benzoquinone compounds were larger with increasing alkyl-side-chains at position 6 of quinone nucleus, and it is clear that the principal product in the reaction mixture is the desired (XI)-acetate. So, the reaction mixture was hydrolyzed with alkali and purified by chromatography on Florisil, yielding XI as an orange-yellow oil. The analytical data of XI was not in complete agreement, but it gave a single colored spot in

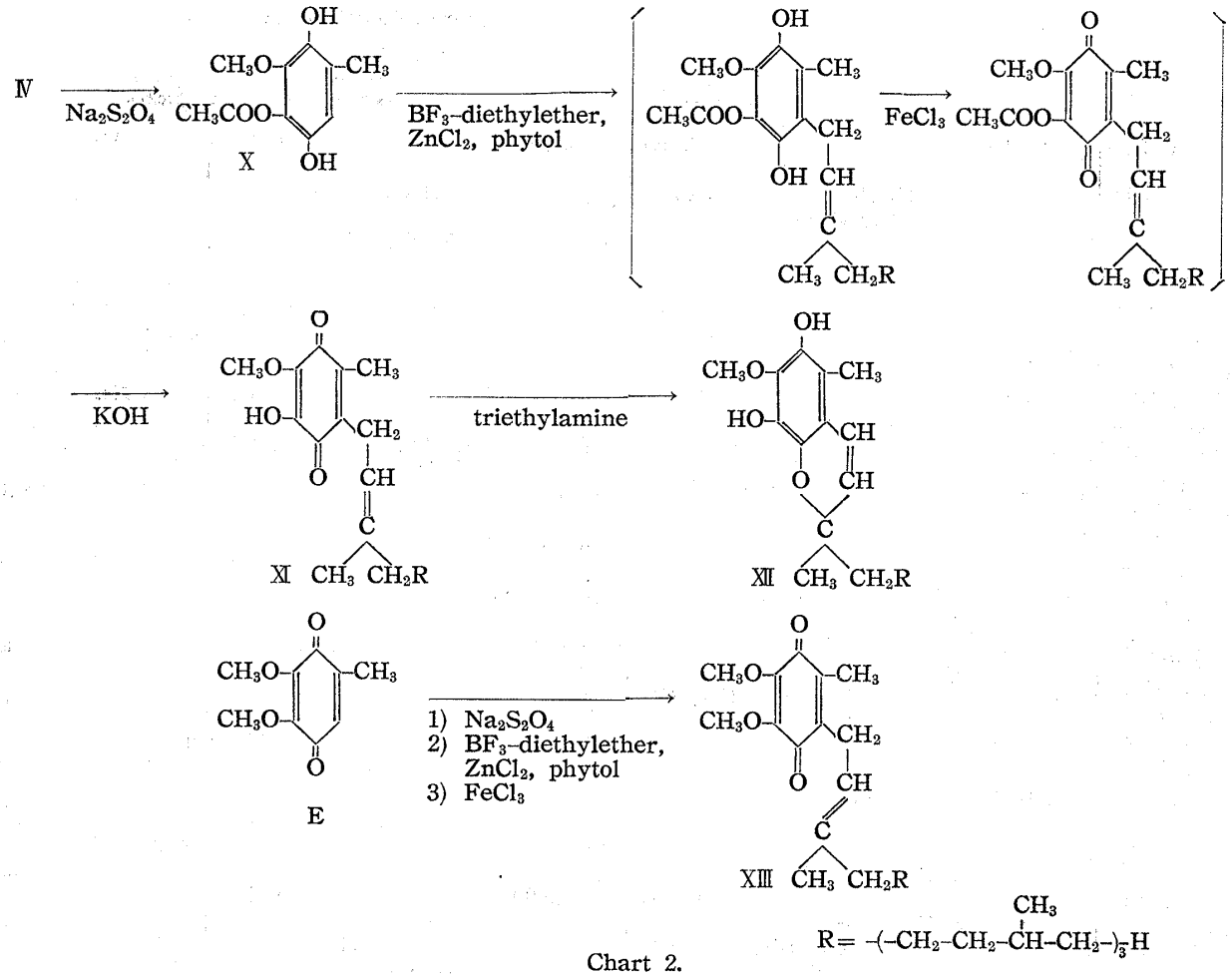

Chart 2.

4) H. Noll, R. Rüegg, U. Gloor, G. Ryser, O. Isler: Helv. Chim. Acta, 43, 433 (1960).
5) W. K. Anslow, J. N. Ashley, H. Raistrick: J. Chem. Soc., 1938, 439.

thin-layer chromatography(Table II) and its Rf-value was distinctly different from that of the possible 2-hydroxy-3-methoxy-5-methyl-*p*-benzoquinone (IX) and rather akin to that of (B). And as its ultraviolet spectrum showed absorption at 275 mμ (oxidized form) and 350→290 mμ (reduced form), there is no doubt in the validity of the structure of the compound. XI was then heated with triethylamine in a sealed tube according to the authors' method,[2] and the fact that the ultraviolet specutrum of the product (XII) exhibited absorption at 274, 282, 314 mμ characteristic of the chromenol compound also supported the structure. The product was not investigated in detail for its small quantity, but it seemed not to be a stable compound. Unlike (C) reported in the previous paper, XII showed no blue color with phosphomolybdic acid but only in the presence of ammonia. All the results mentioned above powerfully support the structure of demethylubiquinone (35) (B).

Table I. (Solvent system : $CHCl_3$)

Compound	Rf-value
IV	0.35
E	0.37
XI-Acetate	0.67
B-Acetate	0.75
XIII	0.72
A	0.76

Table II. (Solvent system : $CHCl_3$–C_2H_5OH (9:1))

Compound	Rf-value
IX	0.18
XI	0.59
B	0.62

Thin layer : Three grams of Silica Gel G (Merck & Co.) is mixed with 10 ml. of ethylacetate. The suspension is coated on 10 sheets of glass plate (2.5×7 cm.), then the plates are left at 25° for 1 hr.
Coloring reagent : Leucomethylene blue reagent.[6]

Experimental*3

Synthesis of 2-Acetoxy-3-methoxy-5-methyl-*p*-benzoquinone

3-Methoxy-4-acetoxybenzaldehyde (I)——According to the method of Pschorr, *et al.*,[3] a solution of vanillin (m.p. 83°, 50 g.) in *N* KOH (330 ml.) was shaken with a solution of acetic anhydride (45 ml.) in ether (200 ml.) in a separatory funnel. When the red-brown color of the aqueous layer disappeared, the ethereal layer was separated and, after being washed with water and dried over Na_2CO_3, was concentrated *in vacuo* to give I, which was purified by recrystallization from dil. ethanol in colorless needles, m.p. 77° (61.2 g., 97.0%), (literature m.p. 77°).

2-Nitro-3-methoxy-4-acetoxybenzaldehyde (II)——According to the method of Pschorr, *et al.*,[3] I (10.2 g.) was added to HNO_3 (d=1.52, 30 ml.) under cooling with ice water and stirring. After keeping at 0~5° for 1 hr. the reaction mixture was poured on ice water (1 L.) and the separated nitro-compound was recrystallized from C_2H_5OH–H_2O (1:1) to give colorless needles, m.p. 87° (7.2 g.. 57.1%), (literature m.p. 85~87°).

2-Nitro-3-methoxy-4-isopropoxybenzyl Alcohol (III)——To a solution of II (46 g.) in iso-PrOH (400 ml.) was added aluminum isopropoxide (40 g.) and the resulting acetone was distilled off. The mixture was then gradually distilled for 10 hr. under addition of iso-PrOH (total 1200 ml.) until the distillate became negative to Legar reaction, and the aluminum isopropoxide was decomposed with 10% HCl (200 ml.). The procuct was extracted with ether and the residue (46 g.) of the ether solution was recrystallized from diluted ethanol to give colorless needles, m.p. 123° (46 g., 99.1%). *Anal.* Calcd. for $C_{11}H_{15}O_5N$: C, 54.76; H, 6.27. Found : C, 54.67; H, 6.29.

2-Acetoxy-3-methoxy-5-methyl-*p*-benzoquinone (IV)——i) A solution of II (10 g.) in C_2H_5OH (100 ml.) was catalytically reduced in the presence of 10% Pd-C (10 g.), when 3700 ml. of H_2 was absorbed (3820 ml. as 5 equivalents of H_2). The reaction mixture was evaporated to dryness *in vacuo*, and to a solution of the residue (8 g.) in 25% H_2SO_4 (125 ml.) was added gradually to a solution of $Na_2Cr_2O_7$ (5 g.)in H_2O (30 ml.) under cooling with ice water and stirring. After stirring the mixture for 12 hr. two times the

*3 Melting points are uncorrected.

6) 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).

volume of the $Na_2Cr_2O_7$ solution was further added and left standing for 3 hr. The reaction mixture was extracted with ether and a solution in hexane (5 ml.) of the residue of the ether solution was adsorbed on a column (3×18 cm.) packed with Florisil (50 g.). The column was eluted with hexane–$CHCl_3$ (8:2), the eluate was investigated by thin-layer chromatography (solvent system : $CHCl_3$, coloring reagent : Leucomethylene blue), and the residue of the Ⅳ-containing fraction was recrystallized from ligroin to give yellow needles, m.p. 48°(500 mg., 5.7%). UV λ_{max}^{EtOH} mμ ($E_{1cm}^{1\%}$) : 265 (618), 358 (37.5). IR ν_{max}^{KBr} cm^{-1} : 1780, 1195 (CH_3CO); 1665, 1620 (quinone). *Anal.* Calcd. for $C_{10}H_{10}O_5$: C, 57.14; H, 4.80. Found : C, 56.95 H, 4.56.

ii) A solution of Ⅷ (500 mg.) in C_2H_5OH (50 ml.) was stirred occasionally in the presence of Raney Ni (5 g.) at 20° for 10 days. The reaction mixture was evaporated to dryness *in vacuo* and a 1% ethanolic $FeCl_3$ solution (50 ml.) was added to the residue. After the mixture was left standing for 12 hr., H_2O (50 ml.) was added and extracted with ether. The extract was poured on a column (2×2 cm.) packed with Florisil (2 g.) and eluted with hexane–$CHCl_3$ (1:1) (100 ml.) to give Ⅳ (10 mg., 3.7%).

2-Nitro-3-methoxy-4-hydroxybenzaldehyde (V)——According to the method of Pschorr, *et al.*,[3] a suspension of Ⅱ (7.2 g.) in H_2O (70 ml.) was warmed with 33% NaOH (14ml.) to make a clear solution. The solution was neutralized with 10%HCl and the separated (V) was recrystallized from water, m.p. 139° (5.5 g., 92.7%), (literature m.p. 137°(corr.)).

3-Methoxy-4-hydroxy-2,5-dinitrobenzaldehyde (VI)——A solution of V (14.4 g.) in acetic acid (250 ml.) was added dropwise to HNO_3 (d=1.50, 5 ml.) at 15°. After stirring for 1 hr., the reaction mixture was poured into ice water (500 ml.) and the separated crystals were recrystallized from $CHCl_3$ to afford colorless needles, m.p. 94°(11.4 g., 64.4%). *Anal.* Calcd. for $C_8H_6O_7N_2$: C, 39.68; H, 2.50; N, 11.57. Found : C, 39.65; H, 2.61; N, 11.68.

3-Methoxy-4-hydroxy-2,5-dinitrobenzaldehyde Diethyl Thioacetal(VII)——Ⅵ (28.3 g.) was added to a mixture of C_2H_5SH (180 ml.), $ZnCl_2$ (16 g.) and Na_2SO_4 (20 g.) under cooling with ice water and left standing for 17 hr. in a cool place. After standing for additional 5 hr. at 20°, the reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in petr. ether and left standing to give yellow plates, m.p. 64°(40.7 g., 99.9%). *Anal.* Calcd. for $C_{12}H_{16}O_6N_2S_2$: C, 41.37; H, 4.63; N, 8.04. Found : C, 41.91; H, 4.65; N, 7.72.

3-Methoxy-4-acetoxy-2,5-dinitrobenzaldehyde Diethyl Thioacetal (VIII)——A solution of Ⅶ (20.2 g.) in acetic anhydride (100 ml.) was added dropwise to pyridine (1 ml.), and after standing for 30 min. at room temperature the reaction mixture was poured into ice water (1 L.) to separate yellow plates, m.p. 53.5°(22.4 g., 99.0%). *Anal.* Calcd. for $C_{14}H_{18}O_7N_2S_2$: C, 43.07; H, 4.65; N, 7.17. Found : C, 43.16; H, 4.65; N, 6.94.

2-Hydroxy-3-methoxy-5-methyl-*p*-benzoquinone (IX)——To a solution of Ⅳ (10 mg.) in C_2H_5OH (1 ml.) was added 30% ethanolic KOH (0.5 ml.) with stirring well, and the mixture, after neutralization with 10% HCl and addition of H_2O (1.5 ml.), was extracted three times with 10 ml.-portions of ether. The extract was evaporated to remain a yellow oil, which gave violet color with the magnesium acetate reagent.[7] IR ν_{max}^{liquid} cm^{-1} : 3400 (OH), 1650, 1620 (quinone) no 1780, 1195 (CH_3CO) observed in Ⅵ.

Synthesis of 2-Hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone

2-Acetoxy-3-methoxy-5-methylhydroquinone (X)——To a solution of Ⅳ (600 mg.) in C_2H_5OH–ether (1:1) (60 ml.) was added dropwise 10% $Na_2S_2O_4$ (60 ml.) in an atmosphere of N_2 under addition of small pieces of dry ice. The reaction mixture was extracted with three 50 ml.-portions of ether and the extract was washed with water and evaporated to dryness to leave a gray-white powder (590 mg., 97.5%).

2-Hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone (XI)——To a solution of X (310 mg.) in dioxane were added BF_3–diethylether (0.3 ml.) and $ZnCl_2$ (500 mg.), and a solution of phytol (1 ml.) in dioxane was added in an atmosphere of N_2 over a period of 30 min. with stirring. After stirring for additional 30 min., the reaction mixture was poured into ice water (100 ml.) and extracted with three 50 ml.-portions of ether. The extract was dissolved in 1% ethanolic $FeCl_3$ solution (50 ml.) and oxidized by stirring for 12 hr. and H_2O (50 ml.) was added and again extracted three times with 50 ml.-portions of ether. The ether solution was evaporated to dryness *in vacuo* to leave a yellow oil which was a mixture (196 mg.) consisting of 2-acetoxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone, the unreacted X and excess phytol. A solution of the mixture in C_2H_5OH (10 ml.) was hydrolized with 30% KOH (1 ml.), neutralized with 10% HCl and extracted with three 50 ml.-portions of ether. The extract was dissolved in hexane–$CHCl_3$ (1:1) (1 ml.), the solution was poured on a column (1×10 cm.) packed with Florisil (3 g.) and the column was washed with C_2H_5OH–hexane (1:1) and eluted with CH_3OH–haxane (1:1) to give a violet solution of XI, which turned yellow by addition of 3*N* HCl (0.5 ml.). The solution was extracted three times with 50 ml. of ether and the ethereal extract was evaporated to dryness *in vacuo* to leave XI as an orange-colored oil (84 mg., 12.9%), UV λ_{max}^{EtOH} mμ : oxidized form 275; reduced form 350→290.

7) S. Shibata : Yakugaku Zasshi, **61**, 320 (1941).

2-(4,8,12-Trimethyltridecyl)-2,5-dimethyl-7-methoxy-2*H*-benzo[*b*]pyran-6,8-diol (XII)——XI (10 mg.) was heated with triethylamine (0.1 ml) in a sealed tube in an atmosphere of N_2 at 100° for 1 hr. The reaction mixture was evaporated with benzene to dryness *in vacuo* and the residue was dissolved in $CHCl_3$ (0.1 ml.). The solution was applied on a thin layer (2.5×7 cm.) of silicagel (0.4 g.) and developed with $CHCl_3$–C_2H_5OH (9:1). The portion showing a slightly higher Rf-value than XI and positive to $FeCl_3 \cdot K_3Fe(CN)_6$-reaction[8] was scraped off and extracted with three 5 ml.-portions of ethanol. The yellow oil obtained by evaporating the extract scarcely exhibited coloration on filter paper by spraying with phosphomolybdic acid but showed a distinct blue-color in NH_3. UV λ_{max}^{EtOH} mμ: 274, 282, 314.

5-Methyl-6-phytyl-2,3-dimethoxy-*p*-benzoquinone (XIII)——To a solution in C_2H_5OH-ether (1:1) (20ml.) of the 5-methyl-2,3-dimethoxy-*p*-benzoquinone (E) (850 mg.) prepared by the method of Anslow, *et al.*[5] was added dropwise 10% $Na_2S_2O_4$ (10 ml.) in an atmosphere of N_2. H_2O (30 ml.) was added to the reaction mixture and extracted with three 100 ml.-portions of ether, and the ether solution was evaporated to dryness *in vacuo* to yield the corresponding hydroquinone compound (800 mg.) as white needles. A solution of the product (100 mg.) in dioxane (10 ml.) was treated with $ZnCl_2$ (1 g.), BF_3-diethylether (0.6 ml.) and phytol (0.6 ml.)as in the case of XI and the resulting 5-methyl-6-phytyl-2,3-dimethoxyhydroquinone was oxidized with 1% ethanolic $FeCl_3$ solution to yield crude XIII. The crude product was dissolved in hexane (2 ml.), adsorbed on a column (1.4×26 cm.) packed with silicic acid • Hyflo Super-cel (2:1) (12 g.) and eluted with hexane–$CHCl_3$ (9:1). The eluate was investigated by thin-layer chromatography (solvent system : $CHCl_3$, coloring reagent : Leucomethylene blue), and the fraction containing the desired compound was evaporated to dryness *in vacuo* to give XIII as an orange-colored oil (88 mg., 32.8%). UV λ_{max}^{EtOH} mμ: oxidized form 273; reduced form 290. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.35; H, 10.77.

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Summary

2-Acetoxy-3-methoxy-5-methyl-*p*-benzoquinone (IV) was synthesized by two methods starting from vanillin but the yield was not good in either case. The hydroquinone compound of IV was condensed with phytol and the product was hydrolyzed to give 2-hydroxy-3-methoxy-5-methyl-6-phytyl-*p*-benzoquinone (XI). Treatment of XI with triethylamine afforded the chromenol compound (XII), and difference in color reaction with phosphomolybdic acid of demethylubichromenol (35) (C) derived from demethylubiquinone (B) gave a powerful support to the structure of the latter (B).

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8) G. M. Barton, R. S. Evans, J. A. F. Gardner : Nature, **170**, 249 (1952).