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Syntheses and Activities of Bioquinone Substances. II.[1] Ubichromenol Phosphates and Related Compounds

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The syntheses of ubichromenol phosphates (III, $n=3$, 6 and 9) and related compounds are described. Their chromatographic and spectral data were given. Moreover, III ($n=9$) was hydrogenated to perhydroubichromenol phosphate (IV) which was also prepared through the two different ways starting from ubiquinone-(45) II($n=9$).

From the results of a preliminary study of III ($n=9$) on the mitochondrial respiration of rat liver, it was found that the succinate dehydrogenase activity was not affected, but both the succinate oxidase and the succinate–neotetrazolium chloride oxidoreductase activities were inhibited, and a marked difference from the effect of ubiquinone.

It is well known that ubiquinone (II) does play an important role in the electron transport system of organism. On the other hand, the biological implication of ubichromenol (I), the isomer of II, seems to be obscured as yet, while it was confirmed that I could not be substituted for II *in vitro*[3] and also did not converted to II *in vivo*.[4,5] Recent studies have shown that the phosphate of chromanol, the structure of which resembled closely to that of I, may be a key intermediate in the oxidative phosphorylation.[6–8] In view of the above situation, it was of interest for us to investigate ubichromenol phosphate (III) in conjunction with its function on the oxidative phosphorylation. This paper reports on the synthesis of III and biological data.

The synthesis was carried out as follows (Chart 1).

Ubichromenol-(45) (I, $n=9$), obtained by heating ubiquinone-(45) (II, $n=9$) with triethylamine at 100—110° in a sealed tube[9] was phosphorylated with phosphorus oxychloride in pyridine and the product, thus obtained, was purified by silica gel column chromatography giving ubichromenol-(45) phosphate (III, $n=9$). Although III ($n=9$) showed single spot on paper (PC) and thin-layer chromatography (TLC) and its structure was supported by

1) Part I: Y. Watanabe, K. Nakajima, and T. Seki, *Chem. Pharm. Bull.* (Tokyo), **18**, 2208 (1970).
2) Location: *34-1, Takata 3-Chome, Toshimaku, Tokyo.*
3) I. Imada and H. Morimoto, *Chem. Pharm. Bull.*, (Tokyo) **13**, 136 (1965).
4) D.L. Laidman and R.A. Morton, *Biochem. J.*, **83**, 329 (1962).
5) H. Morimoto, I. Imada, and H. Nishida, *Chem. Pharm. Bull.* (Tokyo), **13**, 1164 (1965).
6) R.D. Dallan and J.F. Taylor, *Federation Proc.*, **18**, 210 (1959).
7) I. Chielewska, *Biochem. Biophys. Acta*, **39**, 170 (1960).
8) M. Vilkas and E. Lederer, *Experientia*, **18**, 546 (1962).
9) I. Imada and H. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 1047 (1964).

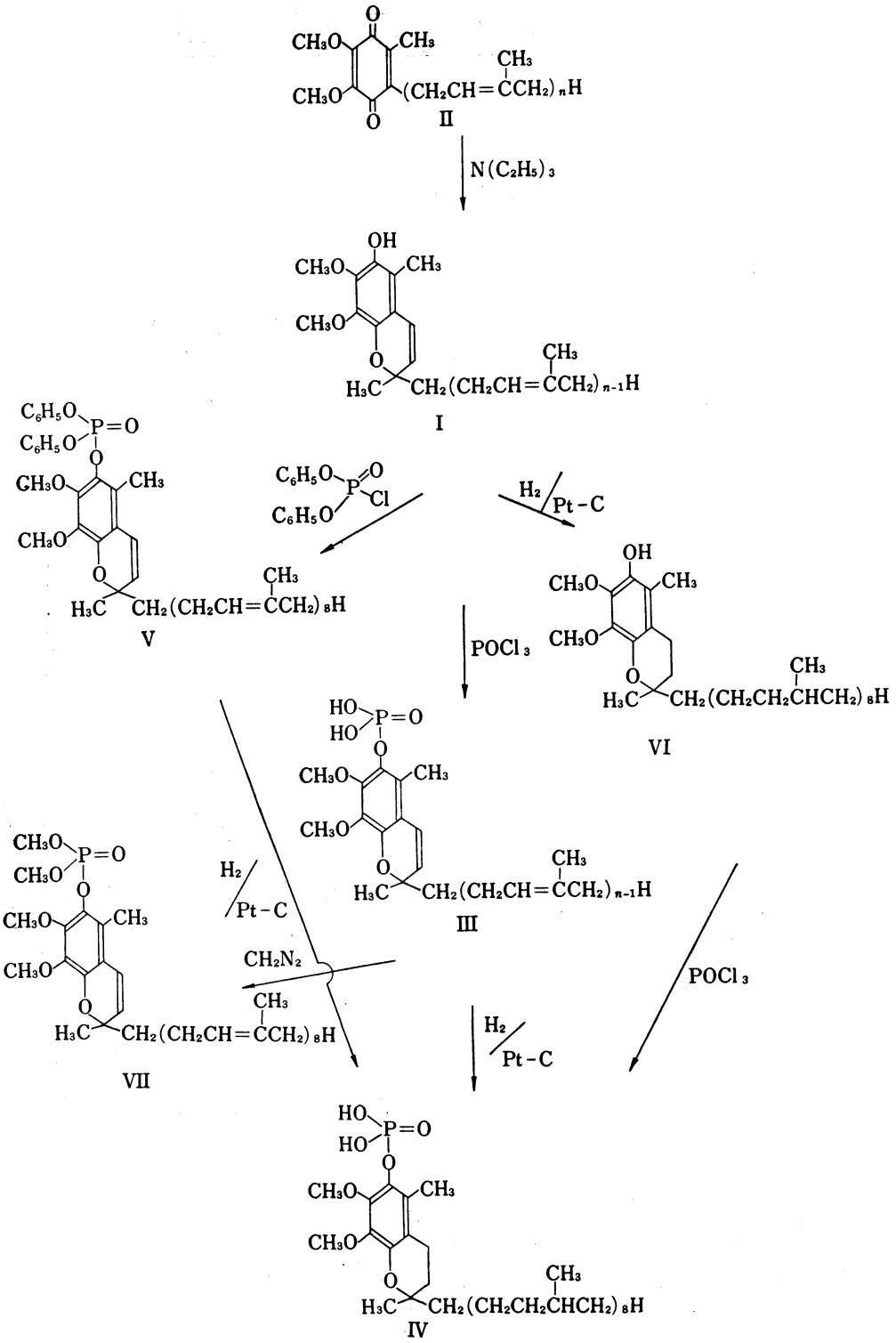


Chart 1

means of ultraviolet (UV) and infrared (IR) spectroscopy and the elemental analysis, its nuclear magnetic resonance (NMR) spectrum indicated only an ill-defined pattern. In order to provide further support for the structure, hydrolysis of III (n=9) to I (n=9) with acid, alkali or phosphatase was carried out, but all attempts were unsuccessful accompanied by a complex decomposition reaction. III (n=9) was catalytically hydrogenated over platinized charcoal (Pt-C) to afford perhydroubichromenol-(45) phosphate (IV), which was also prepared through following processes. Catalytic hydrogenation of I (n=9) over Pt-C gave perhydroubichromenol-(45) (VI), which was phospholyrated with phosphorus oxychloride to yield the desired (IV). Another synthesis of IV was performed by reaction of I (n=9) with diphenyl phosphorochloridate[10] in pyridine and hydrogenolysis of the resulting ubichromenol-(45) diphenylphosphate (V) over Pt-C. Independently of the synthetic routes, there was obtained the same (IV), being confirmed by UV, IR spectroscopy and chromatographic methods. From the above experimental fact, the structure of III (n=9) was undoubtedly established.

III (n=9) was methylated with diazomethane in ether to afford ubichromenol-(45) dimethyl phosphate (VII).

IV, V, and VII showed single spot on PC and TLC respectively, however, the NMR spectrum of IV indicated a ill-resolved signal as like as that of III (n=9), presenting a striking

TABLE I

Compound	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	P	Found C	H	N	P
III, n=3	40.9	$C_{24}H_{35}O_7P$	61.79	7.56		6.64	61.27	7.74		6.17
III, n=6	66.1	$C_{39}H_{59}O_7P$	69.82	8.86		4.62	70.47	9.00		4.04
III, n=9	85.0	$C_{54}H_{83}O_7P$	74.11	9.56		3.54	73.29	9.53		3.55
III, n=9 Ammonium salt	78.0	$C_{54}H_{86}O_7NP$	72.69	9.72	1.57		72.32	10.12	1.80	
IV	62.2	$C_{54}H_{101}O_7P$	72.60	11.40		3.47	71.92	11.25		3.57
V	60.6	$C_{66}H_{91}O_7P$	77.16	8.93		3.01	77.59	8.55		3.05
VII	38.8	$C_{56}H_{87}O_7P$	74.46	9.71		3.43	74.22	9.79		3.05
VIII	37.6	$C_{29}H_{49}O_7P$	64.42	9.14		5.73	63.59	9.13		5.35

TABLE II. Paper Chromatography of Ubichromenol Phosphates and Related Compounds

Compound	*Rf* Benzene–CH_3OH (1:1)	$CHCl_3$–CH_3OH (1:1)
III, n=3	0.89	0.87
III, n=6	0.90	0.88
III, n=9	0.91	0.89
III, n=9 Ammonium salt	0.92	0.89
IV	0.71	0.67
V	0.92	0.88
VII	0.92	0.91
VIII	0.89	0.90

Toyo-Roshi No. 51 was used and carried out by ascending method.
Spots were detected by iodine vapour.

10) P. Brigl and H. Müler, *Ber.*, **72**, 2121 (1939).

Table III. Thin-Layer Chromatography of Ubichromenol Phosphates and Related Compounds

Compound	Rf[a]		Rf[b]	
	Benzene–*n*-BuOH (6:4)	$CHCl_3$–*n*-BuOH (6:4)	Benzene–EtOH (8:2)	$CHCl_3$–EtOH (95:5)
III, $n=3$	0.87	0.89		
III, $n=6$	0.91	0.91		
III, $n=9$	0.90	0.89		
IV	0.78	0.82		
V			0.89	0.75
VII			0.75	0.51
VIII	0.88	0.89		

a) Plate coated with 0.05 mm thickness of microcrystalline cellulose (Avicel SF) was dried overnight at room temperature and then activated at 80° for 20 min.
b) Plate coated with 0.2 mm thickness of silica gel (Wakogel B-O) was dried overnight in air and then activated at 80° for 2 hr.
Spot was detected by iodine vapour.

Table IV. The Spectral Characteristics of Ubichromenol Phosphates and Related Compounds

Compound	UV $\lambda_{max}^{isooctane}$ [a] mμ	IR ν_{max}^{neat} [b] cm^{-1}		
		P-OH	P=O	P-O-P
III, $n=3$	320, 276, 285(s), 230(s)	2700(b)	1280	1060, 1030
III, $n=6$	320, 276, 285(s), 230(s)	2700(b)	1280	1060, 1030
III, $n=9$	320, 276, 285(s), 230(s)	2700(b)	1280	1060, 1030
III, $n=9$[c] Ammonium salt	312, 277, 284(s)	2700(b)	1280	1070, 1040
IV	284, 226(s)	2700(b)	1265	1060, 1030
V	318, 276, 285(s), 232		1290	1180, 1050, 1040
VII	318, 276, 285(s)		1280	1190, 1050, 1040
VIII	320, 276, 285(s), 230(s)	2700(b)	1280	1060, 1030

a) The UV spectra were determined with a Hitachi EPS-2U apparatus.
b) The IR spectra were recorded on a Hitachi IR Spectrometer.
c) EtOH was used as a solvent.
s: shoulder, b: broad

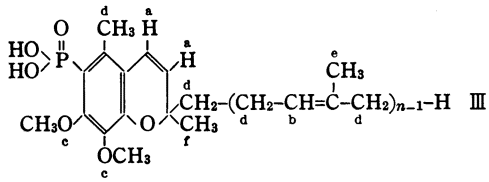
contrast to the spectra of V and VII which showed sharp signals. Similarly, the phosphates of ubichromenol-(15), ubichromenol-(30) and phytylubichromenol were synthesized by reaction of corresponding ubichromenols with phosphorus oxychloride. The NMR spectrum of each compound showed almost the same pattern as that of III ($n=9$). The characteristics of NMR spectra of ubichromenol phosphates and related compounds were summarized in Table V.

Preliminary Study on the Biological Effect of III ($n=9$)

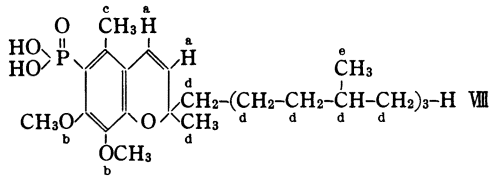
III ($n=9$) ammonium salt was used throughout the experiment. In a striking contrast to the effect of ubiquinone, III ($n=9$) did not recover the succinate oxidase activity of acetone treated mitochondria,[11] but rather it inhibited the activity. Therefore, the effect of III ($n=9$) on intact rat liver mitochondria was examined using a Warburg apparatus. As shown in Table VI, III ($n=9$) accelerated slightly the succinate oxidase activity at the lower concentration (0.05 mM), while it inhibited the activity at the higher concentration. This inhi-

11) S. Okui, Y. Suzuki, and K. Momose, *J. Biochem.*, **54**, 471 (1963).

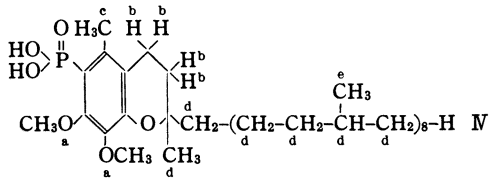
TABLE V. The NMR Characteristics of Ubichromenol Phosphates and Related Compounds[a)]



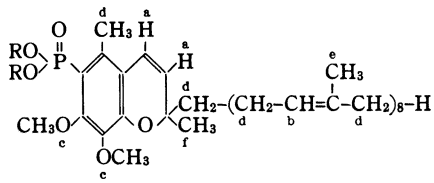
Compound	a	b	c	d	e	f
$n=3$	3.52, 4.50 (1H, d; 1H, d) $J=10$	5.0 (2H, t)	6.32 (6H)	7.9—8.2 (11H)	8.3—8.6 (9H)	8.8 (3H, s)
$n=6$	3.75, 4.75 (1H, d; 1H, d) $J=10$	5.12 (5H, t)	6.4 (6H)	7.8—8.3 (23H)	8.3—8.63 (18H)	8.75 (3H, s)
$n=9$	3.52, 4.5 (1H, d; 1H, d) $J=10$	4.9 (8H, t)	6.2 (6H)	7.9—8.25 (35H)	8.35—8.5 (27H)	8.62 (3H, s)



Compound	a	b	c	d	e
VIII	3.70, 4.68 (1H, d; 1H, d) $J=10$	6.35 (6H)	7.95 (3H, s)	8.2—9.0 (24H)	9.0—9.3 (12H)



Compound	a	b	c	d	e
IV	6.28 (6H, s)	7.5 (4H)	8.0 (3H, s)	8.2—9.0 (59H)	9.0—9.3 (27H)



Compound	a	b	c	d	e	f	R
V (R=C_6H_5)	3.55, 4.77 (1H, d; 1H, d) $J=11$	4.90 (8H, t)	6.19, 6.23 (3H, s; 3H, s)	7.8—8.1 (35H)	8.35—8.4 (27H)	8.65 (3H, s)	2.6—2.8 (10H)
VII (R=CH_3)	3.56, 4.51 (1H, d; 1H, d) $J=10$	4.95 (8H, t)	6.1 (6H, s)	7.8—8.1 (35H)	8.3—8.5 (27H)	8.63 (3H, s)	4.25, 4.28 (3H, d; 3H, d) $J_{POCH}=6$

a) NMR spectra were obtained using a Hitachi Perkin-Elmer R-20 and samples were dissolved in CCl_4 containing tetramethylsilane as an internal standard. J was represented as cps value.
S: singlet, d: doublet, t: triplet
Chemical shifts were reported in τ values.

bition increased with increasing concentration of III ($n=9$),as well as with decreasing the content of mitochondrial protein.

III ($n=9$) did not affect the succinate dehydrogenase activity (acceptor: phenazine methosulfate) at the concentration ranging 0.005—50 mM.

TABLE VI. Effect of (III,$n=9$) Ammonium Salt on the Succinate Oxidase Activity (μl O_2/40 min)[a]

Mitochondrial protein (mg)	(III, $n=9$) (mM)				
	0	0.05	0.1	0.5	1.0
7.6	160	185 (+9.4)	145 (−9.4)	68 (−57.5)	26 (−83.8)
10.6	150	168 (+12.0)	156 (+4.0)	116 (−22.7)	54 (−64.0)

a) The incubation mixture consisted of 0.25 M sucrose, 10 mM KCl, 10 mM phosphate buffer (pH 7.4), 0.2 mM disodium EDTA, 2.5 mM $MgCl_2$, 0.05 M sodium succinate and mitochondrial preparation. pH: 7.4, final volume: 3.0 ml, temperature: 30°, gas phase: air, preincubation: 3—5 min, center well: 20% KOH (0.2 ml)
Figure in parenthesis showed percent increase (+) or decrease (−).

TABLE VII. Effect of (III, $n=9$) Ammonium Salt on the Succinate–Neotetrazolium Chloride Oxidoreductase Activity[a]

Protein content of mitochondrial preparation (mg/tube)	Relative activity (%)	Protein content of mitochondrial preparation (mg/tube)	Relative activity (%)
0.82	32	2.54	67
1.82	47	5.20	82
1.90	58		

a) The incubation mixture consisted of 0.1 M phosphate buffer, 0.2 mM disodium EDTA, 10 mM Tris-HCl and mitochondrial preparation.
total volume: 1.1 ml, pH: 7.4, temperature: 37°, neotetrazolium chloride: 0.5 mg (0.2 ml solution), preincubation (without the acceptor): 5 min, incubation: 10 min, determination of formazan at E_{530} after being extracted with ethyl acetate (5.0 ml).

The succinate–neotetrazolium chloride oxidoreductase activity was inhibited by III ($n=9$) at the same concentration range as that noticed by the succinate oxidase inhibition (>0.1 mM). When mitochondria containing 1.4 mg of protein was used, the inhibition increased according as the concentration of III ($n=9$) increased. As shown in Table VII, the inhibition of 1.0 mM III ($n=9$) increased with decreasing the content of mitochondrial protein. These results support the assumption that the site of inhibition of III ($n=9$) may be located between primary dehydrogenase and cytochrome c of the electron transfer chain in mitochondria.

Experimental

All products were pale yellow viscous liquids. Solvents were removed *in vacuo*. Column chromatography: i) Compounds (III, IV and VIII); silica gel "Wakogel Q-23" (Wakojunyaku Co., Ltd.), ii) Compounds (V, VI, and VII); silica gel "Wakogel C-200" (Wakojunyaku Co., Ltd.).

Preparation of Ubichromenols (I, $n=3$, 6, and 9) and Phytylubichromenol——These compounds were synthesized according to the procedure described by Imada, *et al.*[9,12]

12) Each compound was characterized by TLC on Silica gel G (Merck) with i) $CHCl_3$, ii) benzene and iii) benzene–EtOH (9:1), IR and NMR spectroscopy.

I, $n=3$: Yield, 63%. *Anal.* Calcd. for $C_{24}H_{34}O_4$: C, 74.58; H, 8.87. Found: C, 74.87; H, 8.79.
I, $n=6$: Yield, 50%. *Anal.* Calcd. for $C_{39}H_{58}O_4$: C, 79.28; H, 9.89. Found: C, 79.39; H, 9.84.
I, $n=9$: Yield, 65%. *Anal.* Calcd. for $C_{58}H_{82}O_4$: C, 81.56; H, 10.39. Found: C, 81.74; H, 10.38.
Phytylubichromenol: Yield, 50%. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.39; H, 10.50. Found: C, 75.68; H, 10.43.

Preparation of Ubichromenol Phosphates (III, $n=3$, 6 and 9) and VIII. General Procedure——To a stirred solution of freshly distilled phosphorus oxychloride (0.037 mole) in dry pyridine (5 ml) was added dropwise a solution of the corresponding ubichromenol (0.0037 mole) in the same solvent (6 ml) at −10—−5°, and stirring was continued at room temperature for 3 hr. The resulting solution was poured into ice-water. After being acidified with 10% HCl, the mixture was extracted with ether. The ethereal extract was washed with water and dried over anhyd. Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel (50 g). Elution with *n*-hexane gave the corresponding phosphate.

The analytical results were summarized in Table I. Their characterization were performed by PC and TLC ,and UV, IR and NMR spectroscopy (Table II—V).

Ammonium Salt III ($n=9$)——III, ($n=9$, 1.4 g) was dissolved in 20 ml of MeOH–ether (1:1) and NH_3 gas was saturated under cooling. After evaporation of the solvent, there was obtained III ($n=9$) ammonium salt (1.1 g). The results of analysis and characteristics were given in Table I—V.

Perhydroubichromenol-(45) (VI)——A solution of I, ($n=9$, 2.5 g) in EtOH (35 ml) was hydrogenated over 5% Pt-C at room temperature until no more hydrogen was absorbed. The catalyst was removed by filtration and the filtrate was concentrated and dissolved in a small amount of *n*-hexane. The solution was poured on a column of silica gel (50 g), developed with *n*-hexane, *n*-hexane–$CHCl_3$ (9:1) and *n*-hexane–$CHCl_3$ (7:3) successively. Elution with *n*-hexane–$CHCl_3$ (7:3) gave 1.5 g (58.6%) of VI.[12] *Anal.* Calcd. for $C_{54}H_{100}O_4$: C, 79.74; H, 12.39. Found: C, 80.08; H, 12.47. NMR (CCl_4) τ: 4.88 (1H, singlet, OH), 6.13 (3H, singlet, OCH_3), 6.28 (3H, singlet, OCH_3), 8.0 (3H, singlet, ring-CH_3), 7.5 (4H), 8.2—8.9 (59H), 9.0—9.5 (27H).

Perhydroubichromenol-(45) Phosphate (IV)——A) III ($n=9$, 1.1 g) was hydrogenated by shaking with 5% Pt-C in EtOH–cyclohexane (1:1) (50 ml) at room temperature until there was no absorption of hydrogen. After collection of the catalyst and removal of the solvent, the remaining oil was purified by column of silica gel (20 g). Elution with *n*-hexane gave 700 mg (62.2%) of IV. The results of analysis and characteristics were given in Table I—V.

B) This compound was also obtained from VI in the yield of 91.1% by the same procedure as described for the preparation of III ($n=9$). It was identical with the product obtained above in all respects.

C) V (600 mg), the preparation of which was mentioned below, in EtOH–cyclohexane (1:1) (40 ml) was shaken with hydrogen over 5% Pt-C. After working up by the same procedure as described in the method A), there was obtained 200 mg (38.3%) of IV, which was identical with the sample obtained above in all respects.

Ubichromenol-(45) Diphenyl Phosphate (V)——To a stirred solution of freshly distilled diphenyl phosphorochloridate (6 g) in dry pyridine (5 ml) was added a solution of III ($n=9$, 5.8 g) in the same solvent (10 ml). The reaction mixture was then heated at 100° with stirring for 5 hr. The cooled solution was poured into ice-water. After being acidified with 10% HCl, the mixture was extracted with ether. The ethereal solution was washed with water and dried over anhyd. Na_2SO_4. After evaporation of the solvent, the oily substance was chromatographed on silica gel (80 g), developed with *n*-hexane, *n*-hexane–$CHCl_3$ (9:1) and *n*-hexane–$CHCl_3$ (7:3) successively. The starting material was first eluted from the column and the pale yellow-colored band was then eluted with *n*-hexane–$CHCl_3$ (7:3) giving 4.5 g of V. The analytical result and characteristics were shown in Table I—V.

Ubichromenol-(45) Dimethyl Phosphate (VII)——A solution of III ($n=9$, 1 g) in ether (10 ml) was added with stirring to an ethereal solution of diazomethane prepared from N-nitroso-N-methylurea (5 g) at −5°. The resultant solution was further stirred at 0° for 2 hr. After removal of the solvent, the residual syrup was chromatographed on silica gel (20 g). Elution with *n*-hexane–$CHCl_3$ (6:4) gave 400 mg of VII. The results of analysis and characteristics were given in Table I—V.

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