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A New Phosphorylating Reagent. IV.[1,2] The Preparation of the Mixed Phosphoric Diesters of *dl*-α-Tocopherol and Ethylene Glycol Analogues by Means of 2-Chloromethyl-4-nitrophenyl Phosphorodichloridate

Yoshitaka Mushika and Naoto Yoneda

Chemical Research & Development Laboratory, Tanabe Seiyaku Co., Ltd.[3]

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The mixed phosphoric diesters (**2**) of *dl*-α-tocopherol and ethylene glycol analogues, such as diethylene glycol, its monomethyl ether, pentaethylene glycol and polyethylene glycol 600, were conveniently prepared by two routes shown in Chart 2 and 3 by using 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**). Moreover, diethylene glycol bis (*dl*-α-tocopheryl hydrogen phosphate) (**11**) was prepared and the structures of **2** were confirmed by comparison with **11**. It was observed that the mixed esters **2** derived from pentaethylene glycol and polyethylene glycol 600 were easily soluble in water.

Several investigations of preparing water-soluble derivatives of α-tocopherol involving alkaline metal[4,5] and amine salts[5,6] of α-tocopheryl phosphate (**1**) have been published. It has been also reported by Nakagawa, *et al.*[7] that the mixed phosphoric diester (**2**), which is derived from *dl*-α-tocopherol and polyethylene glycol 600 (an average molucular weight is approximately 600), is soluble not only in alkaline but also in acidic water. However, the isolation of **2** from the reaction mixture was very difficult because of the complicate reaction of *dl*-α-tocopheryl phosphorodichloridate with monosodium polyethylene glycolate.

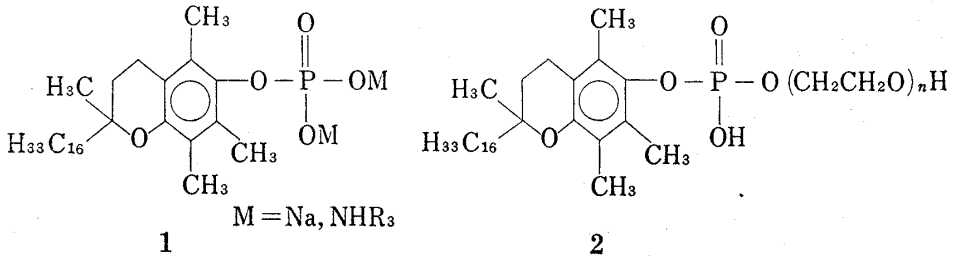


Chart 1

In the previous paper,[2] a convenient preparation method for the mixed dialkyl hydrogen phosphate was demonstrated by using 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**), which was a new phosphorylating reagent having an "activatable protecting group," as starting material.

The present work describes the successful application of this method for the preparation of a few mixed phosphoric diesters (**2**) of *dl*-α-tocopherol and ethylene glycol analogues.

The mixed diester **2** was prepared by the sequence of reactions (route A) shown in Chart 2. The phosphorodichloridate **3** reacted with one mole of *dl*-α-tocopherol in the presence of

1) This work was presented at the 22th Anual Meeting of Chemical Society of Japan, Tokyo, Apr. 1969.
2) Part III: Y. Mushika, T. Hata and T. Mukaiyama, *Bull. Chem. Soc. Japan*, **44**, 232 (1971).
3) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) P. Karrer and G. Bussmann, *Helv. Chim. Acta*, **23**, 1137 (1940).
5) U. V. Solmssen and J. Lee, U. S. Patent 2457932 (1949) [*C. A.*, **43**, 4702e (1949)].
6) O. Ishisaka and K. Murakami, Japan Patent 1737 (1962) [*C. A.*, **58**, 3400d (1963)].
7) T. Nakagawa and Y. Mori, *Yakugaku Zasshi*, **75**, 1322 (1955).

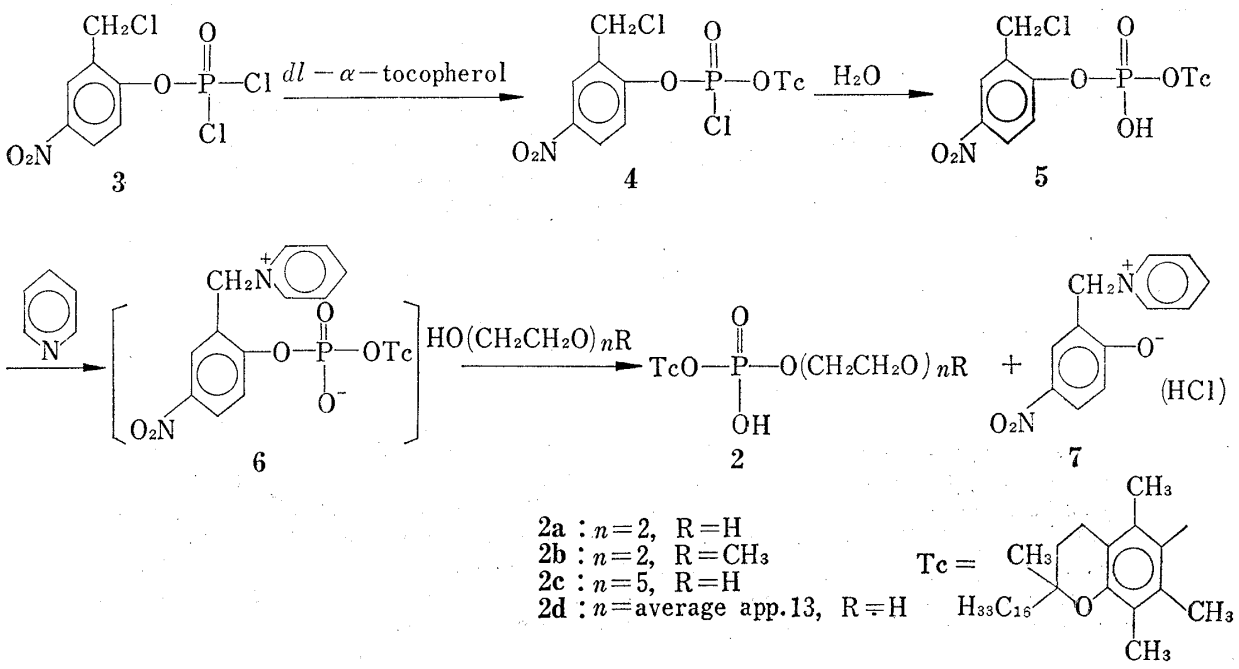


Chart 2 (Route A)

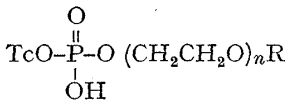
one mole of pyridine in tetrahydrofuran solution to give *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphorochloridate (**4**) which was readily hydrolyzed to afford *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (**5**). The phosphate **5** was easily isolated from the reaction mixture by extraction with benzene and purified by silicagel column chromatography to afford the pure **5** in 84% yield based on **3** as pale brown viscous oil. The structure of **5** was supported by elementary analysis, infrared (IR) and ultraviolet (UV) spectra. Furthermore, **5** was confirmed by converting into *dl*-α-tocopheryl phosphate (**1**) which was identified with an authentic specimen, synthesized according to Karrer's method,[4] by comparison with their IR and UV spectra.

The phosphate **5** was readily derived into an inner salt of 1-[2'-(*dl*-α-tocopheryl hydrogen phosphoroxy)-5'-nitro]benzyl pyridinium hydroxide (**6**), an active phosphorylating reagent, by treating with a large excess of anhydrous pyridine at room temperature for about 2 days. The inner salt **6** phosphorylated ethylene glycol analogues, such as diethylene glycol, its monomethyl ether, pentaethylene glycol[8] and polyethylene glycol 600, to give the corresponding mixed phosphoric diester **2** along with 1-(2'-hydroxy-5'-nitro)benzyl pyridinium chloride (**7**) which was identified with an authentic sample[9] by comparison of their IR spectra. The phosphorylation of the second hydroxy group of ethylene glycol analogues was dodged by the use of an excess amounts of glycol. The phosphate **2** was isolated in the form of pyridinium salt since the phosphorylation of glycols with the intermediate **6** was carried out in pyridine solution. The pyridinium salt was detected by the presence of three weak maximum absorptions of UV spectrum in ethanolic solution in the region of 250 and 265 mμ. The pyridine–free phosphate **2** was obtained by treating with Amberlite IR 120 resin (H form). UV spectra in ethanolic solution of the phoshates **2**, thus obtained as a pale yellow viscous liquid, showed a maximum absorption at 288 mμ and a shoulder at 281.5 mμ and were closely resembled to characteristic bands of α-tocopheryl acetate.[10] The structures of 2 were also supported by elementary analyses and their IR spectra. The results are listed in Table I.

8) R. Goto, N. Koizumi, N. Hayama, T. Sugano and Y. Hirai, *Bull. Inst. Chem. Research, Kyoto Univ.*, **30**, 43 (1952).
9) T. Hata, Y. Mushika and T. Mukaiyama, *J. Am. Chem. Soc.*, **91**, 4532 (1969).
10) W. H. Sebrell and Jr. R. S. Haries, "The Vitamins, Chemistry, Physiology and Pathology," Vol. III, Academic Press, Inc., New York, 1954, p. 497.

The mixed esters **2** were also obtained by a different combination of reactions (route B) as indicated in Chart 3.

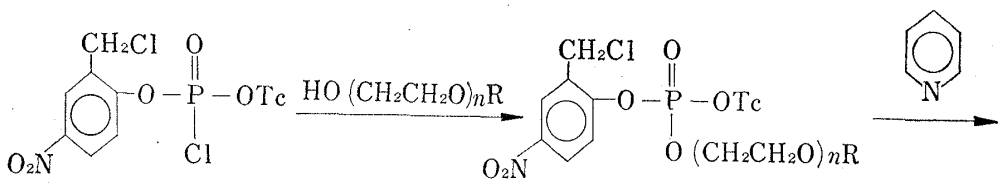
TABLE I. Polyoxyethylene *dl*-α-Tocopheryl Hydrogen Phosphates (2)



Compd.[a]	Synthetic[b] route	Yield[c] (%)	UV: λ_{max}^{EtOH} mμ (log ε)	IR: ν_{max}^{film} cm^{-1} OH	IR: ν_{max}^{film} cm^{-1} P=O
2a	A	58	288 (3.33)	3290	1240
	B	63	281 (shoulder)		
2b	A	54	288 (3.33)	—	1240
	B	77	281 (shoulder)		
2c	A	56	288 (3.35)	3350	1247
	B	90	281 (shoulder)		
2d	A	40	288 ($E_{1cm}^{1\%}$ 36.2)	3410	1247
			281 (shoulder)		

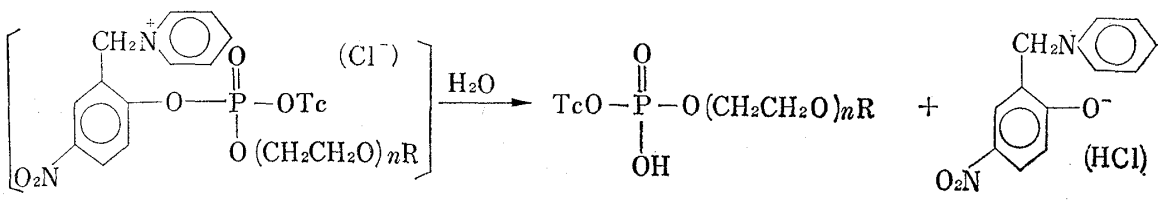
Compd.[a]	*Rf*[d]	Formula	Elementary analysis (%) Calcd. C	H	P	Found C	H	P
2a	0.89	$C_{33}H_{59}O_7P \cdot {}^1/_2H_2O$	65.21	9.95	5.10	64.86	9.95	5.13
2b	0.89	$C_{34}H_{61}O_7P \cdot {}^1/_2H_2O$	65.67	10.05	4.98	65.29	10.05	4.94
2c	0.87	$C_{39}H_{71}O_{10}P \cdot {}^3/_4H_2O$	62.92	9.56	4.16	62.75	9.79	4.04
2d	0.86							

a) All compounds **2a—d** were pale yellow viscous oils.
b) The synthetic routes were shown in chart 2 and 3.
c) Yields by route A and B were based on *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (**5**) and polyoxyethylene *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphate (**8**), respectively.
d) Paper chromatography was carried out by ascending technique using Toyo-Roshi No. 51A paper [iso-PrOH–conc. NH_4OH–H_2O (7:1:2 v/v)].



4 8

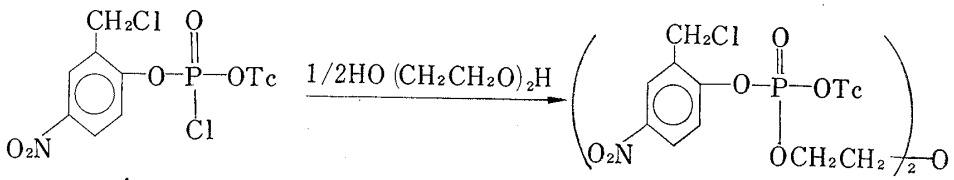
8a : $n=2$, R=H
8b : $n=2$, R=CH_3
8c : $n=5$, R=H



9 2 7

Chart 3 (Route B)

The phosphorochloridate **4**, described above, reacted readily with ethylene glycol analogues, such as diethlene glycol, its monomethyl ether and pentaethylene glycol in the presence of one mole of pyridine to give the corresponding polyoxyethylene *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphates (**8**). In these reactions, the use of 2—3 moles of glycol to **4** was also preferable to avoid the phosphorylation of the second hydroxy group of glycol. When a half mole of diethylene glycol to **4** was employed, its two hydroxy groups were readily phosphorylated with **4** to afford diethylene glycol bis(*dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphate) (**10**) in 53% yield. UV spectra in ethanolic solutions of the phosphoric esters **8**



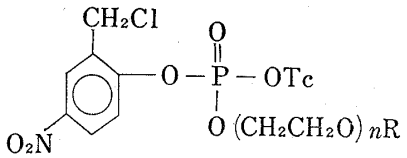
4 **10**

Chart 4

and **10**, showed a maximum absorption at 274 mμ and a shoulder at 288 mμ for characteristic bands of dialkyl 2-chloromethyl-4-nitrophenyl phosphate[2] and α-tocopheryl acylate[10] respectively, but the molar absorptivity of **8** was approximately a half of that of **10**. The identities of the synthesized **8** and **10** were established by elementary analyses and their IR spectra. The results of the phosphoric triesters **8** are shown in Table II.

The triester **8** was readily converted into the desired mixed phosphate **2** through the intermediate, 1-[2′-(polyoxyethylene *dl*-α-tocopheryl phosphoroxy)-5′-nitro]benzyl pyridinium

Table II. Polyoxyethylene *dl*-α-Tocopheryl 2-Chloromethyl-4-nitrophenyl Phosphates (**8**)



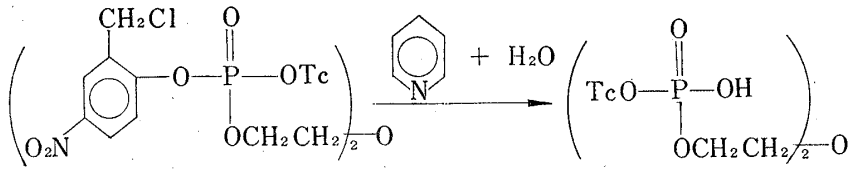
Compd.[a]	Yield[b] (%)	UV: λ_{max}^{EtOH} mμ (log ε)	IR: ν_{max}^{film} cm^{-1} OH	NO_2	P=O	*Rf*[c]
8a	59	274 (3.99) 288 (shoulder)	3410,	1528 1348	1235	0.88
8b	55	274 (3.99) 288 (shoulder)		1528 1348	1235	0.88
8c	53	274 (3.90) 288 (shoulder)	3430,	1528 1348	1238	0.90

Compd.[a]	Formula	Elementary analysis (%) Calcd. C	H	N	P	Found C	H	N	P
8a	$C_{40}H_{63}O_9NClP\cdot{}^1/_2H_2O$	61.80	8.30	1.80	3.98	61.55	8.49	1.68	4.07
8b	$C_{41}H_{65}O_9NClP\cdot{}^1/_2H_2O$	62.22	8.41	1.77	3.91	62.29	8.40	1.94	4.06
8c	$C_{46}H_{75}O_{12}NClP\cdot{}^1/_2H_2O$	60.74	8.42	1.54	3.41	60.95	8.61	1.68	3.30

a) All compounds **8a**,**b** and **c** were pale yellow viscous oils.
b) Yields were based on 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**).
c) Paper chromatography was carried out by ascending technique using Toyo-Roshi No. 51A paper [iso-PrOH–conc. NH_4OH–H_2O (7: 1: 2 v/v)].

chloride (**9**), by treating with a large excess of aqueous pyridine. The mixed phosphates **2**, thus obtained, were identified with **2** prepared according to route A in comparison of *Rf*, UV and IR spectra. The results are summarized in Table I together with ones obtained by route A.

On the other hand, in order to confirm furthermore the structure of **2**, the bis compound **10** was treated with aqueous pyridine in the similar manner as described above. From this reaction, diethyleneglycol bis(*dl*-α-tocopheryl hydrogen phosphate) (**11**), of which properties were apparently different from those of **2**, was obtained together with **7**.



10 **11**

Chart 5

The preparation of **11** was also tried in the manner as shown in Chart 6. A half mole of diethylene glycol was phosphorylated with one mole of the phosphorodichloridate **3** to afford diethylene glycol bis(2-chloromethyl-4-nitrophenyl chlorophosphate) (**12**) which was converted into diethylene glycol bis(2-chloromethyl-4-nitrophenyl hydrogen phosphate) (**13**) by subsequent hydrolysis. When the bisphosphate **13** was treated with a large excess of pyridine at room temperature, a pyridinium derivative **14** precipitated gradually as white crystalline mass which was hardly soluble in usual organic solvents. The compound **14** could not be directly confirmed on account of difficult isolation in pure state, but its structure was supported with the following chemical evidence. When **14** was treated with a large excess of water at 100° for 1 hr, it was broken down to **7** (83%) and diethylene glycol bis(dihydrogen phosphate) (**15**) (53%) which was isolated as white crystal of anilinium salt melting at 160—162°.

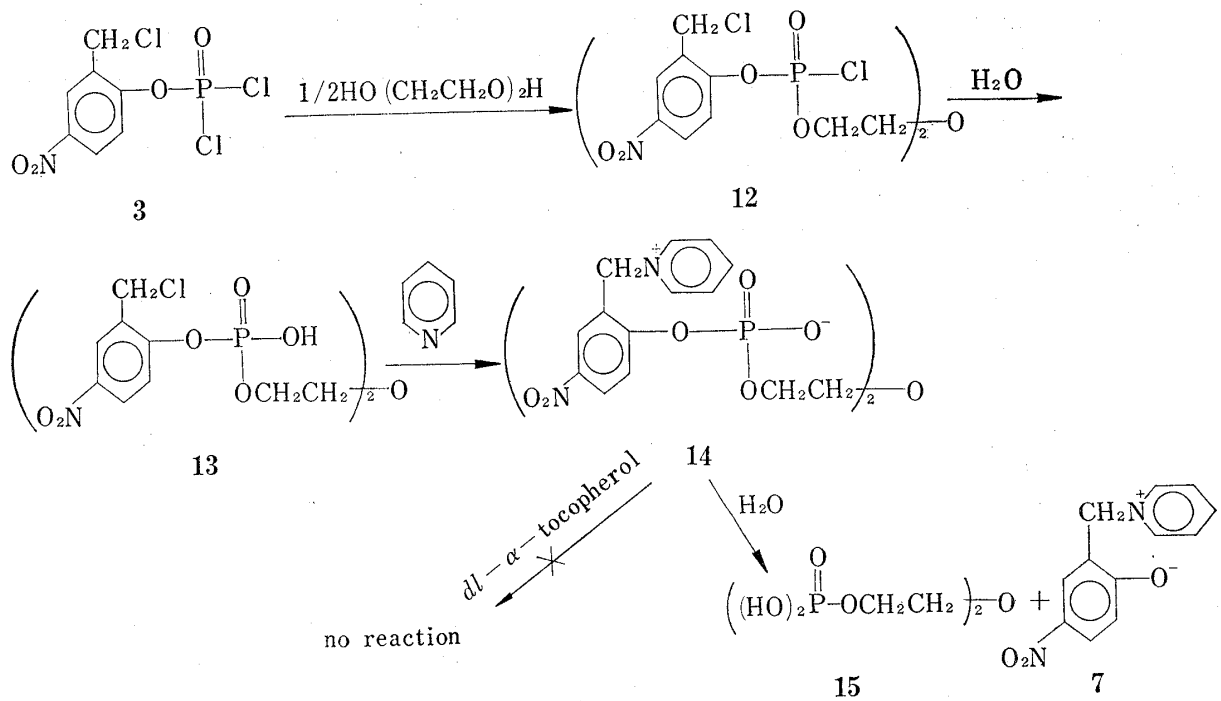


Chart 6

When the phosphorylation of *dl*-α-tocopherol with **14** was tried in the usual manner, both reagents were quantitatively recovered. This is probably rather due to the insolubility of

14 in pyridine than the lower nucleophilicity of α-tocopherol, because even *p*-nitrophenol reacted with inner salt of 1-[2′-(alkyl hydrogen phosphoroxy)-5′-nitro]benzyl pyridinium hydroxide to yield the corresponding alkyl *p*-nitrophenyl hydrogen phosphate.[2)]

The solubility of **2** in neutral water was examined at 22°. The concentration of satutrated aqueous solutions of **2** derived from polyethylene glycol 600, pentaethylene glycol, diethylene glycol and its monomethyl ether were 19.8%, 9.6%, 0.02% and 0.01%, respectively.

In conclusion, the mixed phosphates **2** were successfully prepared by the two routes as shown in Chart 2 and 3 by means of the phosphorodichloridate **3**, the phosphorylating reagent having a new protecting group. Since ethylene glycol analogues except diethylene glycol monomethyl ether have two equal hydroxy groups, it can not be asserted that **2** is the compounds having only one *dl*-α-tocopheryl hydrogen phosphoroxy group. Then, the bis compound **11** was prepared by a suitable combination of reactions and differences between **2** and **11** were examined by comparison of UV and IR spectra, *etc.*

It was observed that the mixed esters **2** derived from pentaethylene glycol and polyethylene glycol 600 were water-soluble derivatives of α-tocopherol.

Experimental

IR spectra were recorded with a Hitachi EPI-S2 IR spectrophotometer. UV spectra were obtained by using a Hitachi EP5-2U recording spectrophotometer. Molecular weights were determined with a Hitachi M-115 molecular weight apparatus. Gas chromatographical analyses were performed on a Hitachi-Perkin Elmer F 6 by using a column of Apiezon L (1.0 m). Paper chromatographies were carried out by ascending technique using Toyo Roshi No. 51A paper. Solvent system used was: isopropanol, conc. ammonia, water (7:1:2 v/v). UV lamp (254 mμ) and Hanes–Isherwood reagent[11)] were used for the detection of spots.

Materials——*dl*-α-Tocopherol was freshly prepared before use by deacetylation of commercially available acetyl *dl*-α-tocopherol. Authentic *dl*-α-tocopheryl phosphate (**1**) was obtained by hydrolysis of the corresponding phosphorodichloridate prepared from *dl*-α-tocopherol and phosphoryl chloride according to Karrer, *et al.*[4)] 2-Chloromethyl-4-nitrophenyl phosphorodichloridate (**3**) was prepared from 2-chloromethyl-4-nitrophenol and phosphoryl chloride according to the previous paper.[9)] Diethylene glycol and its monomethyl ether were purified by distillation. Polyethylene glycol 600 was dried over phosphorus pentoxide in the diminished pressure. Pentaethylene glycol [bp 173—175° (0.12 mmHg)] was separated by several times fractional distillation of commercially available polyethylene glycol 300 and its composition was checked by gas chormatography [12)] and determination of molecular weight.[13)]

***dl*-α-Tocopheryl 2-Chloromethyl-4-nitrophenyl Hydrogen Phosphate (5)**——To a solution of 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**) in 20 ml of tetrahydrofuran (THF) was added a solution of *dl*-α-tocopherol (4.3 g, 0.01 mole) and pyridine (0.8 ml, 0.01 mole) in 20 ml of THF with stirring at 0—10°. The stirring was continued overnight under atmosphere of nitrogen. Then the reaction mixture was poured with stirring into 50 ml of water at a temperature below 15°. After evaporation of THF in the diminished pressure, the residue was extracted with three 30 ml portions of benzene. The combined extracts were washed with water and dried over anhydrous sodium sulfate. After adding 0.8 ml (0.01 mole) of pyridine to the benzene solution, the solution was passed through a column (1.5 × 30 cm) of silicagel (kieselgel 0.05—0.2 mm for column chromatography, manufactured by E. Merck Co., Ltd.) and the column was washed with benzene (a half gram of unreacted *dl*-α-tocopherol was recovered from this washings). Then, the column was eluted with a mixture of chloroform and methanol (4:1 v/v). The eluent was evaporated to dryness under reduced pressure. The syrupy residue was dissolved in 30 ml of benzene and the solution was washed with diluted hydrochloric acid and water. After drying the solution over anhydrous sodium sulfate, the solvent was distilled off *in vacuo* to afford 5.8 g (84%) of the phosphate **5** as pale brown viscous oil.[14)] *Rf* 0.90. UV λ_{max}^{EtOH} mμ (log ε): 289 (4.04), 218 (shoulder). *Anal.* Calcd. for $C_{36}H_{55}O_7NClP \cdot {}^1/_2H_2O$: C, 62.73; H, 8.20; N, 2.03. Found: C, 62.59; H, 8.53; N, 2.51.

11) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
12) Retention times of ethylene glycol and its analogues were: ethylene glycol 10 sec, diethylene glycol 18 sec, triethylene glycol 48 sec, tetraethylene glycol 90 sec, pentaethylene glycol 3 min 24 sec, hexaethylene glycol 8 min 20 sec (under a stream of nitrogen gas (0.5 kg/cm²) at a temperature of 240°).
13) Mol. wt. Calcd. for $HO(CH_2CH_2O)_5H$: 238.3. Found: 239.6.
14) The phosphate **5** was freshly prepared before use since it was gradually colored reddish brown on storing at room temperature. In the following experiments,the crude phosphate **5** was used without purification with silicagel column chromatography.

***dl*-α-Tocopheryl Dihydrogen Phosphate** (1)——A solution of crude *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (5), which was prepared from 1.50 g (0.005 mole) of the phosphorodichloridate 3 and 2.15 g (0.005 mole) of *dl*-α-tocopherol, in a mixture of pyridine (25 ml) and water (0.90 ml, 0.05 mole) was kept at room temperature for 2 days and then heated at 90° for 6.5 hr. After removal of the solvent by distillation *in vacuo*, 20 ml of chloroform was added to the residue. A yellow precipitate, 1-(2′-hydroxy-5′-nitro)benzyl pyridinium chloride (7) (1.05 g, 79.0%) was filtered and washed with 20 ml of chloroform. The combined filtrate and washings were washed with 2% hydrochloric acid and water. After drying the solution over anhydrous sodium sulfate, the solvent was removed by evaporation. The resulted residue was dissolved in 30 ml of benzene and passed through a silicagel column (20 × 200 mm). After washing the column with 200 ml of benzene, the column was eluted with 300 ml of a mixture of chloroform and methanol (4:1 v/v). The eluent was concentrated *in vacuo* to give 2.0 g (78.0% based on 3) of *dl*-α-tocopheryl dihydrogen phosphate (1) as orange red viscous oil. The phosphate **1** was identified with an authentic specimen prepared by Karrer's method[4] in comparison of IR and UV spectra and paper chromatography.

Dioxyethylene *dl*-α-Tocopheryl 2-Chloromethyl-4-nitrophenyl Phosphate (**8a**)——To a solution of 3.04 g (0.01 mole) of the phosphorodichloridate 3 in 20 ml of THF was added, dropwise, a solution of 4.31 g (0.01 mole) of *dl*-α-tocopherol and 0.8 ml (0.01 mole) of pyridine in 15 ml of THF with stirring at a temperature below 0° over a period of about 1 hr. The stirring was continued at room temperature for 10 hr, and then a solution of 3.2 g (0.03 mole) of diethylene glycol and 0.8 ml (0.01 mloe) of pyridine in 15 ml of THF was added to the reaction mixutre in one portion. The stirring was additionally continued at room temperature for 7 hr. After evaporation of THF, the residue was dissolved in 70 ml of benzene. The benzene solution was washed with water and dried over anhydrous sodium sulfate. The solution passed through a column of silicagel (15 × 500 mm). The column was washed with benzene and then was eluted with a mixture of benzene and ethanol (100:5 v/v). After removing the solvent, the residue was dissolved in hexane and decolorized with charcoal. The solvent was evaporated *in vacuo* to afford 4.61 g (59.4%) of the phosphate **8a**.

In the similar way, 2-(2-methoxyethyl) ethyl **8b** and pentoxyethylene derivative **8c** were prepared. The results are listed in Table II.

Dioxyethylene *dl*-α-Tocopheryl Hydrogen Phosphate (**2a**)——Route A: A solution of the crude phosphate **5**, which was prepared from 3.04 g (0.0l mole) of the phosphorodichloridate 3 and 4.31 g (0.01 mole) of *dl*-α-tocopherol, and 3.20 g (0.03 mole) of diethylene glycol in 20 ml of pyridine was allowed to stand at room temperature for 2 days and then heated at 90—95° for 7 hr. After evaporating pyridine *in vacuo*, 30 ml of chloroform was added to the residue. An yellow precipitate, 1-(2′-hydroxy-5′-nitro)benzyl pyridinium chloride (7) (2.24 g, 83.5%) was filtered off and washed with 20 ml of chloroform. After concentration of the combined filtrate and washings to dryness, the resulted residue was dissolved in 50 ml of benzene and passed through a column (15 × 600 mm) of silicagel. The column was washed first with benzene (unreacted *dl*-α-tocopherol was recovered) and next with acetone (an excess of diethylene glycol was recovered). Then, the column was eluted with a mixture of chloroform and methanol (4:1 v/v). The eluent was evaporated to give 4.35 g of pyridinium salt of **2a** as pale brown oil. The oil was dissolved in 99% ethanol and decolorized with charcoal. The ethanolic solution was treated with Amberlite IR 120 resin (H form). From the eluent, 3.55 g (58.6%) of pure **2a** was obtained as pale yellow viscous oil by evaporation in the diminished pressure.

In the similar manner, the corresponding mixed phosphates **2b—d** were prepared from diethylene glycol monomethyl ether, pentaethylene glycol and polyethylene glycol 600. The results are shown in Table I.

Route B: A solution of 3.85 g (0.005 mole) of dioxyethylene *dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphate (**8a**) in a mixture of pyridine (25 ml) and water (5 ml) was kept to stand at room temperature for 2 days and then heated at 90° for 6.5 hr. After concentrating the reaction mixture by evaporation, the residue was treated with 30 ml of chloroform and an insoluble yellow precipitate **7** was filtered off (1.28 g, 96.0%). The filtrate was concentrated to dryness and the oily residue was worked up in similar manner described in route A to afford pure **2a** as pale yellow viscous oil. The mixed phosphate **2a** was identified with an authentic sample prepared by route A by comparison of IR and UV spectra and paper chromatography.

In the similar procedure, the mixed phosphates **2b** and **2c** were synthesized by hydrolysis of the corresponding neutral esters **8b** and **8c**. The results were listed in Table I together with those of route A.

Diethylene Glycol Bis (*dl*-α-tocopheryl 2-chloromethyl-4-nitrophenyl phosphate) (10)——To a solution of 4.56 g (0.015 mole) of the phosphorodichloridate 3 in 20 ml of THF was added a solution of 6.45 g (0.015 mole) of *dl*-α-tocopherol and 1.2 ml (0.015 mole) of pyridine in THF (15 ml) with stirring under cooling. The stirring was continued at room temperature for 10 hr and then a solution of 0.80 g (0.0075 mole) of diethylene glycol and 1.2 ml (0.015 mole) of pyridine in THF (10 ml) was added to the reaction mixture at room temperature in one portion. The stirring was additionally continued for 12 hr. After evaporating THF, the resulted residue was treated with 150 ml of benzene and the benzene solution was washed with water. The solution was dried over anhydrous sodium sulfate and passed through a column (20 × 750 mm) of silicagel. The column was washed with 500 ml of benzene, and then eluted with a mixture of benzene and

ethanol (100:5 v/v). The eluent was collected fractionally (each fraction was 10 g). The fractions[15] No. 7—11 contained pure phosphate **10** and the fractions No. 12—20 contained crude products. The solvent of the combined first fraction (No.7—11) was evaporated to give 3.70 g (34.6%) of the bisphosphate **10** as pale yellow viscous oil and 2 g (18.7%) of **10** was obtained from second fraction (No. 12—20) by further purification with silicagel column chromatography. UV λ_{max}^{EtOH} mμ (log ε): 274 (4.30), 288 (shoulder). IR ν_{max}^{film} cm^{-1}: 1527 and 1349 (NO_2), 1190 (P-O-C_{aryl}), 1040 (P–O–C_{alkyl}), 1110 (broad) and 1080 (C-O-C). *Anal.* Calcd. for $C_{76}H_{116}O_{15}N_2Cl_2P_2$: C, 63.81; H, 8.17; N, 1.96; P, 4.33. Found: C, 63.52; H, 8.75; N, 1.94; P, 4.13.

Diethylene Glycol Bis (*dl*-α-tocopheryl hydrogen phosphate) (11)——A solution of 4.30 g (0.003 mole) of the bisphosphate **10** in a mixture of water (5 ml) and pyridine (20 ml) was kept to stand at room temperature for 2 days and then heated at 95° for 7 hr. The reaction mixture was worked up as the manner described in the preparation of **2a** (route B) to afford 1.62 g (49.4%) of **11** as pale yellow caramel easily soluble in hexane, ether, benzene and chloroform. *Rf* 0.89. UV λ_{max}^{EtOH} mμ (log ε): 288 (3.64), 281 mμ (shoulder). IR ν_{max}^{Nujol} cm^{-1}: 1245 (P=O), 1080 (P-O-C_{aryl}). *Anal.* Calcd. for $C_{62}H_{108}O_{11}P_2$: P, 5.68. Found: P, 5.60.

Diethylene Glycol Bis (2-chloromethyl-4-nitrophenyl hydrogen phosphate) (12)——To a solution of 6.09 g (0.02 mole) of the phosphorodichloridate **3** in 20 ml of THF was added, dropwise, a solution of 1.06 g (0.01 mole) of diethylene glycol and 1.06 ml (0.02 mole) of pyridine in THF (15 ml) with stirring at −20—−10° over a period of about 1 hr. The stirring was continued for 2 hr under cooling and additionally at room temperature for 1 hr. Then, the reaction mixture was poured with stirring into 20 ml of ice–water. THF was evaporated in the diminished pressure to separate viscous oil from aqueous layer. The oily substance was isolated by decantation and washed with two 10 ml portions of water and two 30 ml portions of ether. The oil was dissolved 50 ml of 99% ethanol and decolorized with charcoal. The solvent was removed by evaporation and then dried over phosphorus pentoxide *in high vacuo* at room temperature for about 1 week to give 5.30 g (87.5%) of **13** as pale brown viscous oil. *Rf* 0.88. UV λ_{max}^{EtOH} mμ (log ε): 288 (4.16). *Anal.* Calcd.for $C_{18}H_{20}O_{13}N_2Cl_2P_2$: N, 4.63; P, 10.24. Found: N, 4.52; P, 10.01.

Reaction of Diethylene Glycol Bis (2-chloromethyl-4-nitrophenyl hydrogen phosphate) (13) with *dl*-α-Tocopherol in Pyridine——To a suspension of 3.03 g (0.005 mole) of bisphosphate **13** in 30 ml of pyridine was added 6.47 g (0.015 mole) of *dl*-α-tocopherol. The reaction mixture was stirred at room temperature for 2 days under atmosphere of nitrogen (oily substance changed into crystalline mass) and then heated at 90—100° for 11 hr. After allowing to stand the reaction mixture at room temperature overnight, an insoluble precipitate was filtered and washed with 20 ml of pyridine and 20 ml of ethanol. The combined filtrate and washings were concentrated to dryness in the diminished pressure and the residue was dissolved in 30 ml of benzene. The solution was treated with silicagel column. From the benzene eluent and washings was recovered 6.01 g (93.5%) of unreacted *dl*-α-tocopherol which was identified with an authentic specimen by comparison of IR spectrum and paper chromatography. A solution of the insoluble precipitate in 20 ml of water was refluxed for 1 hr. After concentrating the reaction mixture, the residue was treated with 20 ml of ethanol and an insoluble yellow substance, 1-(2′-hydroxy-5′-nitro)benzyl pyridinium chloride (**7**)(2.20 g, 83.5%) was filtered off. The ethanolic filtrate was concentrated to dryness and the residue was dissolved in 20 ml of water. The aqueous solution was treated with a column of Amberlite IR 120 resin (H form). The acidic eluent was concentrated and the resulted oil was dissolved in 10 ml of ethanol. To the solution was added 2 ml of aniline and the mixture was allowed to stand in a refrigerator overnight to give 1.20 g (53.1%) of the crude dianilinium salt of diethylene bis (dihydrogen phosphate) (**15**). Recrystallization from 95% ethanol afforded white plates for analytical sample. mp 160—162°. *Rf* 0.06. IR ν_{max}^{Nujol} cm^{-1}: 2600—2150 (H_3N^+–), 1225 (P=O), 1050 and 1030 (C-O-P-O^-). *Anal.* Calcd. for $C_4H_{12}O_9P_2 \cdot 2C_6H_5NH_2 \cdot {}^1/_2H_2O$: C, 41.66; H, 5.90; N, 6.07. Found: 41.47; H, 6.04; N, 6.31.

Solubilities of Polyethylene *dl*-α-Tocopheryl Hydrogen Phosphates (2a—d) in Water at 22°——Saturated aqueous solutions of the mixed phosphates **2a, b, c** and **d** containing a small excess of **2** were allowed to stand with occasional shaking and the absorption intensities of the solutions at 288 mμ were determined at inter-

Compd.	Mol. Wt.[a]	Mol. Absorp. (log ε)	Dilutn. Co.	log I_0/I	Conctn., %
2a	607.82	3.33	1	0.858	0.023
2b	621.85	3.33	1	0.425	0.012
2c	744.49	3.35	5×10^2	0.590	9.81
2d		$E_{cm}^{1\%}$ 36.2[b]	2×10^5	0.357	19.76

a) The molecular weights of the hydrated compound **2** are listed.

b) Concentration of **2d** solution was based on $E_{cm}^{1\%}=36.2$.

15) Each fraction was detected by thin–layer chromatography using Kieselgel GF_{254} nach Stahl (E. Merck Co., Ltd.); *Rf* of **10** was 0.91 (solvent: $CHCl_3$–MeOH, 4:1 v/v).

vals (It was observed that the intensities did not change at all after 5 hr). After 7 hr, the definite volume of each solution was diluted with water and its intensity at 288 mμ was determined. The concentration of the original solution was calculated as following; concentration, % (w/v) = Mol. Wt. of $2 \times \log I_0/I \times$ dilutn. coefficient $\times$ 100/molar absorptivity.

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