Synthesis of Lecithin Analogues by Means of Cyclic Enediol Phosphates. Derivatives of 1-Octadecanol and of Cholesterol

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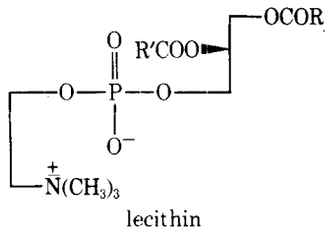
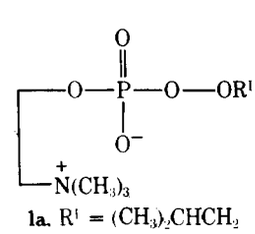
Alkylphosphorylcholines have been synthesized as analogues of the natural phospholipid lecithin (phosphatidylcholine). The synthesis involves three steps: (1) reaction of the lipophilic alcohol, 2-methyl-1-propanol, 1-octadecanol, or cholesterol, with 1,2-dimethylethenylene phosphorochloridate in the presence of triethylamine; (2) triethylamine-catalyzed reaction of the hydrophilic alcohol choline chloride with the alkyl 1,2-dimethylethylene phosphate generated in the first step; and (3) removal of the 1-methylacetonyl blocking group from the alkyl(1-methylacetonyl)phosphorylcholine chloride produced in the second step. The hydrolysis is performed in aqueous acetonitrile, in the presence of triethylamine, and gives the alkylphosphorylcholine zwitterion as a crystalline monohydrate after silica gel chromatography. A second method of synthesis reverses the sequence in which the choline chloride and the lipophilic alcohols are phosphorylated and affords the same alkylphosphorylcholines but in lower yields than the first method.

The phospholipids of biological membranes are phosphodiesters, $(R^{I}O)(R^{II}O)P(O)OH$, derived from a lipophilic and a hydrophilic alcohol. In the natural lipids, the lipophilic moiety, $R^{I}OH$, is a fatty acid ester of glycerol or dihydroxyacetone, or a fatty acid amide of the aminodiol sphingosine.[2] The hydrophilic moiety ($R^{II}OH$), which in conjunction with the phosphate constitutes the polar head group of the molecule, is derived from polyfunctional alcohols such as choline, ethanolamine, serine, *N*-(2-hydroxyethyl)alanine, glycerol, and *myo*-inositol. Lecithin (phosphatidylcholine) is widely distributed in biomembranes and is being extensively employed as the source of the phospholipid bilayer in studies of model membranes by the black film and vesicle techniques.[3] This paper describes the synthesis of several alkyl phosphorylcholines, **1**, in which the diglyceride moiety, $R^{I}OH$, of lecithin is replaced by simpler lipophilic groups. Compounds of this type are being sought for research into the bilayer-forming properties of unnatural phospholipid analogues.



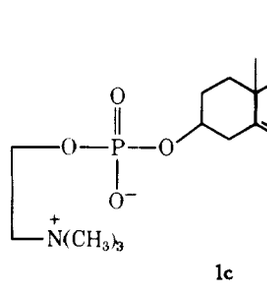
lecithin



1a. $R^{I} = (CH_3)_2CHCH_2$

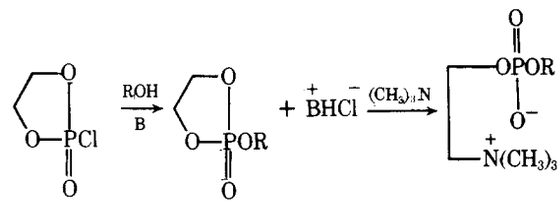
b. $R^{I} = CH_3(CH_2)_{16}CH_2$

One of the lecithin analogues included in the present study is 3-cholest-5-enylphosphorylcholine (**1c**). Cholesterol is present in conjunction with phospholipids in plasma membranes, and it has been suggested that the steroid reduces the area occupied by lecithin at the air–water interface due to mechanical obstruction of the tilting of the lipid molecules.[4] There is evidence that cholesterol influences the packing of phospholipid molecules and the permeability of model membranes made from them.[4] It appeared of interest to replace cholesterol by the "cholesterollecithin", **1c**, in such studies, and also to compare its behavior with that of the recently synthesized optically active[5] and racemic[6] phosphatidylcholesterol, where the steroid is linked to the lipophilic diglyceride group rather than to the hydrophilic choline group. The bilayer-forming properties of these two types of phospholipid analogues are described elsehwere.



1c

Scheme I



Several syntheses of phosphatidylcholine have been reported,[7-11] and an ingenious method to introduce the choline cation into phosphodiesters (Scheme I) has been recently introduced by Chabrier et al.[12] (Scheme I).

The general synthetic method utilized in the present work is summarized in Scheme II.[13,14] The first of the two phosphorus–oxygen bonds in the phosphodiester is established (step 1) by means of a derivative of the 1,2-dimethylethenylenedioxyphosphoryl group, abbreviated X=P(O)–. Either one of the two alcohols being phosphorylated, $R^{I}OH$ (lipophilic) or $R^{II}OH$ (hydrophilic), can play the role of the "first alcohol" or $R^{1}OH$, in the scheme. The second P–O bond is established (step 2) as a result of the amine-catalyzed phosphorylation of the "second alcohol" or $R^{2}OH$ by the alkyl cyclic enediol phosphate generated in step 1. The desired phosphodiester is obtained by hydrolysis of the 1-methylacetonyl (Acn) blocking group. The conversion of the two alcohols into the phosphodiester can be achieved as a "one-, two-, or three-step" syntheses, according to the number of intermediates isolated and purified.

Scheme II[a]

$$R^{1}OH + X{=}P(O)Y + B \rightarrow X{=}P(O)OR^{1} + Y^{-}BH^{+} \quad (1)$$

$$R^{2}OH + X{=}P(O)OR^{1} \rightarrow (R^{1}O)(R^{2}O)P(O)OAcn \quad (2)$$

$$(R^{1}O)(R^{2}O)P(O)OAcn + H_2O \rightarrow (R^{1}O)R^{2}O)P(O)OH + Acn \cdot OH \quad (3)$$

[a] Acn = $CH(CH_3)COCH_3$.

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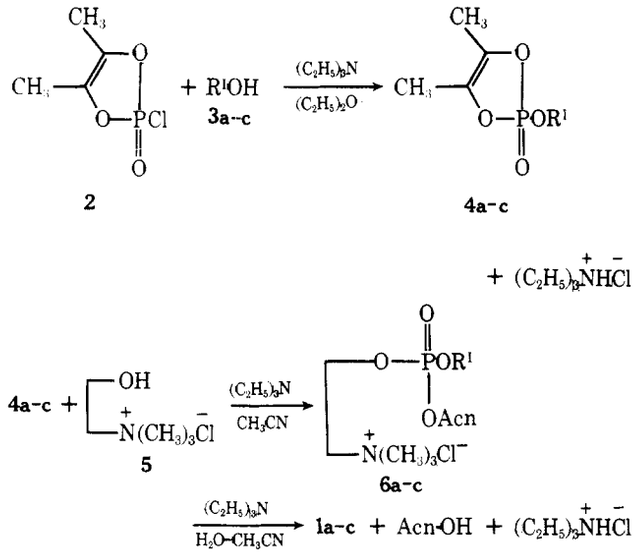
Scheme III[a]



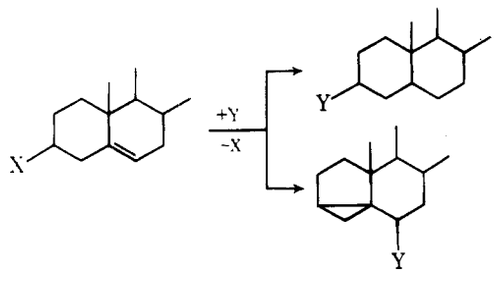
[a] a, $R^I = (CH_3)_2CHCH_2$; b, $R^I = CH_3(CH_2)_{16}CH_2$; c, R^I = 3-cholest-5-enyl; Acn = $CH(CH_3)COCH_3$.

Results

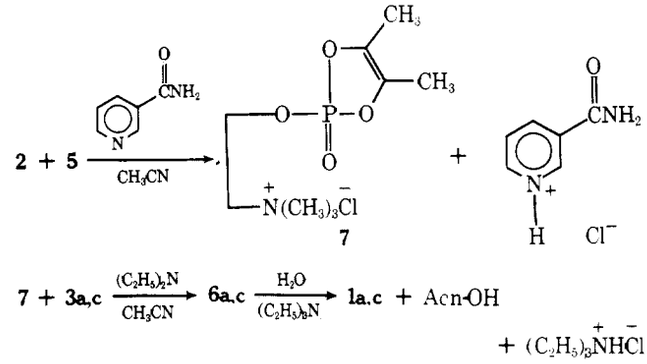
Lipophilic Alcohols (R^IOH) as "First Alcohol" (R^IOH) in the Phosphorylation Sequence. Procedure 1. Three alcohols of increasing lipophilicity, 2-methyl-1-propanol (**3a**), 1-octadecanol (**3b**), and cholesterol (**3c**), are converted into the corresponding alkyl phosphorylcholine zwitterions, **1a–c**, following the sequence of reactions shown in Scheme III. The first step is the phosphorylation of the alcohol by the cyclic enediol phosphorochloridate,[15,16] **2**, and proceeds in virtually quantitative yield; the alkyl cyclophosphate, **4a–c**, is isolated but is not purified. The second step is the phosphorylation of choline chloride (**5**) by the alkyl cyclophosphate, **4a–c**. This reaction yields an acyclic phosphotriester derivative of choline chloride, **6a–c**, which is directly hydrolyzed to the desired alkyl phosphorylcholine zwitterion, **1a–c**. The procedure involves two laboratory operations, since only the alkyl cyclophosphate, **4a–c**, is isolated, although not purified. The zwitterions, **1a–c**, are isolated in 70, 53, and 52% yields, respectively, based on the first alcohol, **3a–c**, after silica gel chromatography. Two of the zwitterions, **1a** and **1c**, are obtained as stable monohydrates, **1a** $\cdot H_2O$ and **1c** $\cdot H_2O$, which retain water even after prolonged drying under vacuum; however, the third zwitterion, **1b**, which contains the octadecyl group is easily dehydrated under comparable conditions.

The structures of the zwitterions, **1a–c**, rest on elemental analysis and ^{31}P and ^1H NMR spectrometry. The structure of 3-cholest-5-enylphosphoryl-choline, **1c**, is also supported by ^{13}C-NMR spectrometry. The key structural features are carbon atoms C3, C5, and C6, and their chemical shifts and multiplicities are those expected from the values found in the parent cholesterol molecule.[17] The ^{13}C parameters of the choline moiety have the values expected from those found for choline chloride.[18] The optical rotation of the zwitterion **1c** is $[\alpha]^{25}_D$ −15.2° (*c* 1.0, CH_3OH), while that of cholesterol is $[\alpha]^{25}_D$ −39.5° (*c* 1.3, $CHCl_3$).

Scheme IV



Scheme V



Scheme VI

$$R^2OH + X{=}P(O)OR^1 \rightarrow X{=}P(O)OR^2 + R^1OH \quad (4)$$

$$X{=}P(O)OR^1 + R^1OH \rightarrow (R^1O)_2P(O)OAcn \quad (5)$$

$$X{=}P(O)OR^2 + R^2OH \rightarrow (R^2O)_2P(O)OAcn \quad (6)$$

A careful scrutiny of the structure of the zwitterion derived from cholesterol, **1c**, is mandatory in view of the occurrence of the *i*-cholesterol rearrangement (Scheme IV) during nucleophilic displacements at C3 of certain cholesterol derivatives.[19,20] It is apparent that this rearrangement does not play a significant role in the preparation of the alkyl cyclophosphate, **4c**, and in the subsequent phosphorylation of choline chloride (**5**) by the phosphate, **4c**, at least under the specified conditions, in spite of the relatively high-energy content of the alkyl cyclic enediol phosphate, **4c**.

Hydrophilic Alcohol ($R^{II}OH$ = Choline Chloride) as "First Alcohol" (R^IOH) in the Phosphorylation Sequence. Procedure 2. The flexibility of the present phosphodiester synthesis in the field of lecithin analogues would be increased by the utilization of choline chloride in the first step of the phosphorylation sequence. This procedure has been utilized in the alternate preparation of 2-methyl-1-propyl- and 3-cholest-5-enylphosphorylcholine **1a** and **1c**, as shown in Scheme V.[21]

Although this procedure 2 is feasible, the zwitterions are obtained in significantly lower yields than in procedure 1, e.g., 43 and 37% for **1a** and **1c**, respectively. The reason for this decrease in efficiency is shown in Scheme VI. A nucleophilic displacement with ring retention, instead of ring opening, in the second step of the synthesis (eq 4 in Scheme VI) decreases the yield of the desired *unsymmetrical* dialkyl-1-methylacetonyl phosphate (eq 2 in Scheme II), since the transesterification reaction permits the formation of undesirable *symmetrical* dialkyl-1-methylacetonyl phosphates (eq 5 and 6, Scheme VI). In one case, substantial amounts (ca. 17%) of one of these symmetrical triesters, di-(2-methyl-1-propyl)-1-methylacetonyl phosphate, was isolated in the corresponding synthesis according to procedure 2.

Experimental Section

Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Reactions involving derivatives of the 1,2-dimethylethenylenedioxyphosphoryl group must be carried out under strictly anhydrous conditions. Choline chloride was recrystallized from anhydrous ethanol and was kept 24 h at 20 °C (0.2 mm). Cholesterol was anhydrous grade. δ ^{31}P in ppm vs. H_3PO_4 = 0; positive values are down-field from the reference compound; τ ^1H in ppm vs. Me_4Si = 10; ^1H-decoupled δ ^{13}C in ppm to low field of Me_4Si = 0; all spectra at ~30 °C.

2-Methyl-1-propylphosphorylcholine Monohydrate (1a· H_2O). Procedure 1. A solution of 2-methyl-1-propanol (2.13 g, 28.8 mmol)

and triethylamine (2.19 g, 1 molar equiv) in diethyl ether (20 mL) was added dropwise to a stirred ether solution (80 mL) of 1,2-dimethylethenylene phosphorochloridate[15b] (**2**; 4.85 g, 28.8 mmol) at 20 °C After 2 h at 20 °C, the alkylammonium salt was filtered and washed with ether (3 × 20 ml). The combined ether solution was evaporated to yield the cyclic phosphate **4a** (6.2 g, 100% yield, after 1 h at 20 °C (0.2 mm)). A solution of the cyclic phosphate **4a** (4.82 g, 28.2 mmol) in acetonitrile (100 mL) was treated with choline chloride (3.94 g, 28.2 mmol, added at once), followed by triethylamine (7.86 mL, 2 molar equiv), at 20 °C. The heterogeneous mixture was stirred for 24 h at 20 °C, at which point it had become homogeneous and contained the alkyl-1-methylacetonylphosphorylcholine chloride, **6a**. Water (200 mL) was added to the acetonitrile solution, and the mixture was stirred for 24 h at 20 °C. The solution was evaporated at 40 °C (20 mm, and 0.2 mm), the residue was dissolved in chloroform (20 mL), and the solution was applied to a column (4 × 52 cm) containing silica gel 60 (400 g, Merck Catalogue No. 7734, packed in $CHCl_3$). Elution with $CHCl_3$ (2 L) and with mixtures of $CHCl_3/CH_3OH$ (99/1, 2 L; 95/5, 2 L; 90/10, 2 L; 80/20, 2 L; 50/50, 1.5 L) removed impurities. The alkyl phosphorylcholine, **1a**, appeared in 4 L of methanol; the solvent was evaporated and the residue was kept 12 h at 20 °C (0.2 mm) to give 4.03 g (70%) of **1a**. ^{1}H-NMR spectra (in CD_3OD) revealed that this material could be nearly anhydrous or could contain variable amounts of water depending on the degree of exposure of the sample to atmospheric moisture. The zwitterion, **1a**, was converted into its monohydrate by addition of moist acetone (20 mL) to a methanol (5 mL) solution of the chromatographed product (4.0 g). The crystals were filtered and dried for several hours at 20 °C (0.2 mm) to yield **1a**·H_2O, mp 225–230 °C (with decomposition after losing water at ca. 120 °C); δ ^{31}P = −0.6 ppm (CD_3OD); τ ^{1}H = 9.05 (doublet, J = 7 Hz), 8.00, 6.72 (singlet) 6.32, 5.70, and 5.11 (singlet) ppm in CD_3OD. Anal. Calcd for $C_9H_{22}O_4NP·H_2O$: C, 42.0; H, 9.4; N, 5.4; P, 12.0; H_2O, 7.0. Found: C, 42.2; H, 9.5; N, 5.4; P, 12.0; H_2O, 6.9 (K. Fischer method).

1-Octadecylphosphorylcholine (1b). Procedure 1. This compound was prepared by the procedure described above, with the following variations. From 1-octadecanol (6.52 g) and triethylamine (2.44 g) in diethyl ether (20 mL), and the phosphorochloridate **2** (4.06 g) in diethyl ether (100 mL), there was obtained the cyclic phosphate **4b** as a white solid. From **4b**, choline chloride (3.29 g), and triethylamine (4.77 g) in acetonitrile (500 mL), after stirring for 36 h at 20 °C, there was obtained a solution of the alkyl 1-methylacetonylphosphorylcholine chloride, **6b**. The solution was evaporated at 30 °C (30 mm), the residue was dissolved in water (200 mL) and acetonitrile (100 mL), and the mixture was treated with triethylamine (4.77 g) and stirred for 10 h at 70 °C. The residue obtained after evaporation was purified by chromatography to give 5.55 g (53%) of **1b**. Crystallization of the crude product (4.26 g) from acetone (30 mL) and methanol (4 mL) gave the *anhydrous* zwitterion **1b**, melting with decomposition at ca. 235 °C (after 12 h at 20 °C (0.2 mm)); δ ^{31}P = 0.0 ppm (CD_3OD); τ ^{1}H = 8.67, 6.70 (singlet), 6.30, and 5.76 ppm in CD_3OD. Anal. Calcd for $C_{23}H_{50}O_4NP$: C, 63.4; H, 11.6; N, 3.2; P, 7.1. Found: C, 63.3; H, 11.9, N, 3.1; P, 7.1; H_2O, 0.6 (K. Fischer method).

3-Cholest-5-enylphosphorylcholine Monohydrate (1c·H_2O). Procedure 1. From cholesterol (4.42 g) and triethylamine (1.16 g) in diethyl ether (20 mL), and the phosphorochloridate **2** (1.93 g) in diethyl ether (100 mL), after 6 h of reaction time at 20 °C, there was obtained the cyclic phosphate **4c** as a white solid, following the procedure described above. A suspension of the cyclic phosphate **4c** in acetonitrile (250 mL) and dichloromethane (250 mL) was treated with choline chloride (1.60 g) and triethylamine (2.32 g) at 20 °C. Reaction time to the alkyl 1-methylacetonylphosphorylcholine chloride, **6c**, was 36 h at 20 °C. The solution was evaporated at 30 °C (30 mm), the residue was dissolved in water (100 mL) and acetonitrile (50 mL), and the mixture was treated with triethylamine (2.31 g) and stirred for 10 h at 70 °C. The residue obtained after evaporation of the resulting solution was purified by silica gel chromatography as described above (the impurities were removed using 3 L of each of the indicated solvent mixtures; the desired product was obtained in 3 L of methanol). The alkyl phosphorylcholine **1c** was obtained as a powder (3.26 g, 52% yield) and was recrystallized from methanol (2 mL) and moist acetone (20 mL). The crystals melted with decomposition at ca. 230 °C after drying for 1 h at 20 °C (0.2 mm) and had the composition of the monohydrate **1c**·H_2O; $[\alpha]^{25}_D$ −15.2° (c 1.0 in CH_3OH); δ ^{31}P = −1.4 ppm (CD_3OD); τ ^{1}H = 9.00, 6.80 (singlet), 6.30, 5.70, and 5.18 (singlet) ppm (CD_3OD); main δ ^{13}C = 76.8 (doublet, J_{COP} = 6 Hz, C3), 122.8 (singlet, C6), and 141.6 (singlet, C5) ppm, in the 3-cholest-5-enyl group, and 54.6 [triplet, J_{CN} = 3.4 Hz, $(CH_3)_3N$], 60.1 (doublet, J_{COP} = 4.9 Hz, CH_2OP), and 67.4 (multiplet, CH_2N) ppm, in the choline group (in CD_3OD). Lit. for cholesterol: δ ^{31}C = 71.0 (C3), 120.9 (C6), and 141.7 (C5) ppm (in pyridine-d_5); Lit. for choline chloride δ ^{13}C = 54.8 (triplet, J_{CN} = 4.1 Hz), 56.6 (singlet, CH_2OH), and 68.3 (triplet, J_{CN} = 3.0 Hz, CH_2N) ppm (in D_2O). Anal. Calcd for $C_{32}H_{58}O_4NP·H_2O$: C, 67.5; H, 10.6; N, 2.5; P, 5.4; H_2O, 3.2. Found: C, 67.8; H, 10.7; N, 2.3; P, 5.4; H_2O, 4.6 (K. Fischer method).

1,2-Dimethylethenylenedioxyphosphorylcholine Chloride (7). Choline chloride (5.33 g, 38.2 mmol) was added to a solution of 1,2-dimethylethenylene phosphorochloridate (6.43 g, 38.2 mmol) in acetonitrile (100 mL) containing nicotinamide (4.66 g, 1 molar equiv) in suspension, at 20 °C. The mixture was stirred for 24 h at 20 °C, and was filtered. The filtrate was evaporated (30 °C (20 mm and 0.2 mm)). The residue was dissolved in dichloromethane (100 mL) and was kept for 12 h at −20 °C and filtered to remove the last traces of nicotinamide hydrochloride. The filtrate was evaporated, the residue was redissolved in dichloromethane (100 mL), and the solution was diluted with diethyl ether (20 mL) and kept 12 h at −20 °C. The crystalline choline chloride cyclophosphate ester (**7**) was filtered and dried at 20 °C (0.2 mm). This substance is sensitive to moisture and unlike choline chloride is relatively soluble in dichloromethane and in acetonitrile; it has the following spectral properties: δ ^{31}P = +10.4 ppm ($CDCl_3$); τ ^{1}H = 8.00 (apparent singlet, $CH_3C{=}CCH_3$), 6.42 (singlet), 5.75, and 5.37 ppm ($CDCl_3$).

1,2-Dimethylethenylenedioxyphosphorylcholine 1,2-Dimethylethenylenephosphate. A salt analogous to the chloride **7** was made as described above, but utilizing as reagent bis(1,2-dimethylethenylene) pyrophosphate X=P(O)·O·(O)P=X. The cyclophosphate salt of the choline cyclophosphate ester has the following spectral properties; δ ^{31}P = +10.3 (triplet, J_{POCH_2} = 6.8 Hz), and +11.8 (singlet) ($CDCl_3$); τ ^{1}H = 8.24 (singlet), 8.10 (singlet), 6.60 (singlet), 5.84, and 5.30 ppm ($CDCl_3$).

3-Cholest-5-enylphosphorylcholine Monohydrate (1c·H_2O). Procedure 2. A dichloromethane (20 mL) solution of cholesterol (5.99 g, 15.5 mmol) and triethylamine (3.14 g, 1 molar equiv) was added, dropwise, to a dichloromethane (50 mL) solution of 1,2-dimethylethenylenedioxyphosphorylcholine chloride (**7**; 4.21 g, 1 molar equiv), at 20 °C. The solution was stirred for 20 h at 20 °C and was evaporated. The residue was dissolved in water (100 mL) and acetonitrile (50 mL), and the mixture was treated with triethylamine (3.14 g) and stirred for 16 h at 70 °C. The solution was evaporated, and the residue was purified by column chromatography as described under procedure 1. The alkyl phosphorylcholine **1c** was obtained in 37% yield (3.22 g) and was converted into the monohydrate **1c**·H_2O, mp 230 °C dec, $[\alpha]^{25}_D$ −15.8° (c 1.16, CH_3OH), for characterization.

2-Methyl-1-propylphosphorylcholine Monohydrate (1a·H_2O). Procedure 2. From 2-methyl-1-propanol and the choline chloride cyclophosphate **7**, there was obtained the alkyl phosphorylcholine, **1a**·H_2O, in 43% yield. A by-product of this reaction is di(2-methyl-1-propyl)-1-methylacetonyl phosphate, isolated in 17% yield.

Registry No.—**1a,** 21991-72-0; **1b,** 65956-63-0; **1c,** 65956-64-1; **2,** 21949-38-2; **3a,** 78-83-1; **3b,** 112-92-5; **3c,** 57-88-5; **4a,** 16764-09-3; **4b,** 65956-65-2; **4c,** 65956-66-3; **5,** 67-48-1; **6a,** 65956-67-4; **6b,** 65956-68-5; **6c,** 65956-69-6; **7,** 65956-59-4; 1,2-dimethylethenylenedioxyphosphorylcholine 1,2-dimethylethenylenephosphate, 65956-61-8; bis(1,2-dimethylethenylene) pyrophosphate, 55894-94-5; bis(2-methyl-1-propyl)-1-methylacetonyl phosphate, 65956-62-9;

References and Notes

(1) Research supported by Grant GM-20672 from the National Institute of General Medical Sciences.
(2) G. B. Ansell, R. M. C. Dawson, and J. N. Hawthorne, Ed., "Form and Function of Phospholipids", 2nd ed, Elsevier, Amsterdam, 1973, pp 19, 69, and 128.
(3) D. Papahadjopoulos and H. K. Kimelberg, "Phospholipid Vesicles (Liposomes) as Models for Biological Membranes", Pergamon Press, New York, N.Y., 1973.
(4) Reference 2, pp 136 and 159.
(5) F. Ramirez, P. V. Ioannou, and J. F. Marecek, *Synthesis*, 673 (1977).
(6) T. Muramatsu, I. Hara, and M. Hayashi, *Chem. Phys. Lipids*, **20**, 131 (1977).
(7) E. Baer and A. Kindler, *Biochemistry*, **1**, 518 (1962).
(8) P. E. Verkade, *Bull. Soc. Chim. Fr.*, 1993 (1963).
(9) L. L. M. van Deenen and G. M. De Haas, *Adv. Lipid Res.*, **2**, 167 (1964).
(10) E. Baer, *J. Am. Oil Chem. Soc.*, **42**, 257 (1965).
(11) A. J. Slotboom and P. P. M. Bonsen, *Chem. Phys. Lipids*, **5**, 301 (1970).
(12) N. Hoang-Phuong, N. Thanh-Thuong, and P. Chabrier, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **283**, 229 (1976).
(13) F. Ramirez, J. F. Marecek, and H. Okazaki, *J. Am. Chem. Soc.*, **98**, 5310 (1976).
(14) F. Ramirez and J. F. Marecek, *Acc. Chem. Res.*, in press.
(15) (a) F. Ramirez, H. Okazaki, and J. F. Marecek, *Synthesis*, 637 (1975); (b) F. Ramirez, H. Okazaki, J. F. Marecek, and H. Tsuboi, *ibid.*, 819 (1976).
(16) I. P. Gozman, *Izv. Akad. Nauk SSSR*, 2362 (1968).
(17) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigart, and J. D. Roberts, *J.*

Am. Chem. Soc., 91, 7445 (1969).
(18) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley, New York, N.Y., 1972, p 142.
(19) (a) R. J. W. Cremlyn and N. A. Olsson, J. Chem. Soc. C, 2305 (1969); 2023 (1971); (b) R. J. W. Cremlyn, B. B. Dewhurst, D. H. Wakeford, and R. A. Raja. J. Chem. Soc., Perkin Trans. 1, 1171 (1972).
(20) L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, N.Y., 1959, p 314.
(21) Nicotinamide is used as the proton acceptor in the first step in order to minimize the solubility of the base hydrochloride in the acetonitrile solvent. The latter is used in place of ether to maximize the solubility of choline chloride (5).

Formation of 14α-Cardenolides from 21-Acetoxy-20-keto Steroids

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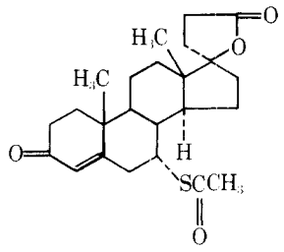
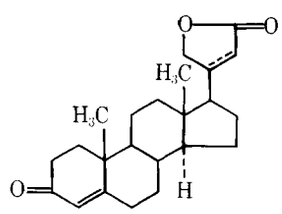
The Emmons–Horner condensation of diethyl cyanomethylenephosphonate with 21-acetoxy-20-keto steroids has been studied. The reaction with 21-acetoxypregnenolone gives a 20-cyanomethylene steroid as a single isomer. The 3-enol ethers of deoxycorticosterone acetate, 11-dehydrocorticosterone acetate, and corticosterone diacetate react similarly. Δ^1-Corticosterone acetate reacts directly with the ylide only at C-20 to form the corresponding 20-cyanomethylene derivative. These modified corticoids undergo ready dehydrogenation to form 1,4-, 4,6-, and 1,4,6-unsaturated ketones. When these cyanomethylene derivatives react with 1 equiv of *p*-toluenesulfonic acid in refluxing aqueous ethanol, transesterification of the 21-acetate and hydrolysis of the nitrile occurs to form a cardenolide ring in high yield. These mild conditions are compatible with a wide variety of other functional groups in the steroid. The corresponding cardanolides have been prepared by ketalization of the 3-ketone and subsequent hydrogenation and deketalization.

We were interested in preparing cardenolides and cardanolides related to the known anti-aldosterone steroid, Spironolactone **1**. If the usual hormonal steroid stereochemistry is introduced into the cardenolides (i.e., C/D-trans) and, additionally, when the requisite 3-keto-4-ene grouping is present, the structural similarity between **2** and **1** becomes apparent.[1]



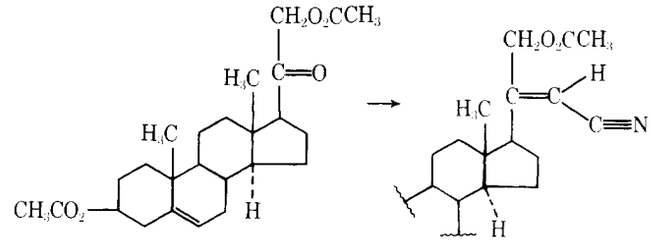
1



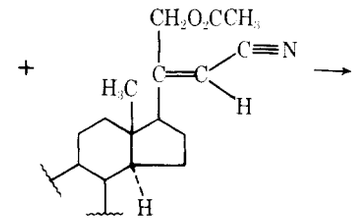
2 ($\Delta^{20,22}$)

31

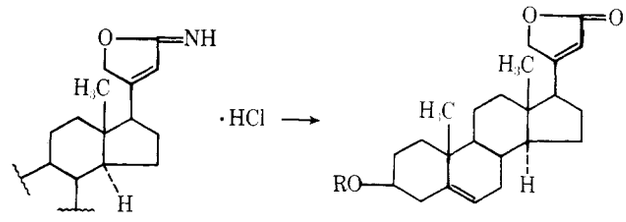
An apparently simpler reaction, proceeding in very high yield, for the formation of the steroid cardenolide ring was described by Fritsch.[2] This involved the reaction of a 21-hydroxy-20-ketone moiety with the anion of trimethyl phosphonoacetate in a modified Horner–Emmons reaction to yield directly the cardenolide ring in yields of greater than 95%.[3] When this reaction was attempted using deoxycorticosterone, we were never able to achieve yields of more than 25%, and this particular reaction appeared to be limited in its applicability. Similar observations were reported by Yoshii and Ozaki on the same condensation with 21-hydroxypregnenolone.[4] During the time that these studies were in progress, Pettit reported on the formation of cardenolides from 21-acetoxy-20-ketones and diethyl cyanomethylenephosphonate.[5] The reaction between 21-acetoxypregnenolone acetate **3** and the Horner–Emmons ylide formed a mixture of 20-cyanomethylene isomers **4** and **6** which were not separated, but the crude reaction mixture was directly reacted with hydrochloric acid to form the iminocardenolide hydrochloride **5** and the pure (*E*)-cyanomethylene isomer **6**. Compound **5** could then be hydrolyzed in refluxing hydrochloric acid to give the cardenolide **7**. The assignment of stereochemistry for **6** was based on repeated



3 **6**



4



5

7. R = $OCCH_3$
8. R = H

unsuccessful attempts to convert it into the cardenolide **7**.[5]

The enol ether **9** of deoxycorticosterone was prepared using standard methods and condensed with the anion of diethyl cyanomethylenephosphonate to give the enol ether **10** which could be hydrolyzed in aqueous acetic acid to the enone **11** in an overall yield of 58%. When basic hydrolysis of the 21-acetate in **11** was attempted only extensive degradation occurred and this approach to the 21-hydroxy compounds was abandoned. As a consequence, acid-catalyzed transesterification

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