SECTION C
Organic Chemistry

Studies of Organophosphorochloridates. Part II.† Reactions of Steroid Phosphorodichloridates

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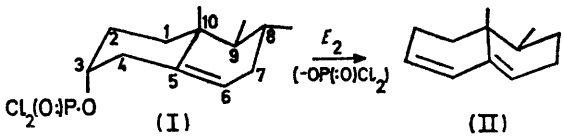
The preparation of the following steroid phosphorodichloridates is described: cholestanyl, epicholestanyl, epicholesteryl, and the derivatives of methyl 3α-hydroxy-5β-cholanate, and ergosta-8(14)en-3β-ol. The formation of the phosphorodichloridates was followed by t.l.c. Attempted phosphorylation of 3,5-cyclocholestan-6β-ol, and 6β-hydroxycholest-4-en-3-ol gave cholesteryl phosphorodichloridate and cholestane 3,6-dione respectively. The hydrolysis of cholesteryl phosphorodichloridate has been examined under various conditions. The steroid phosphorodichloridates have been treated with methanol. Reaction of cholesteryl phosphorodichloridate with a range of alcohols, gave the corresponding cholesteryl ethers. Solvolysis of the phosphorodichloridates by dry pyridine generally gave the corresponding *N*-steroid pyridinium chlorides.

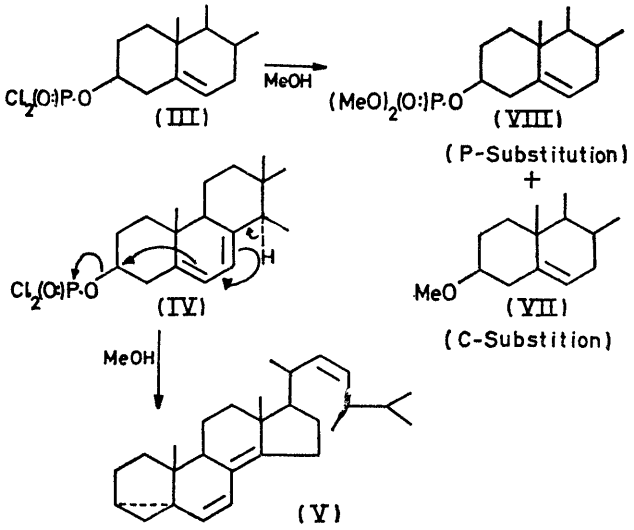
PREVIOUS attempts [1] to convert cholestanol into the phosphorodichloridate with phosphorus oxychloride or pyrophosphoryl chloride were unsuccessful. Cholestanyl phosphorodichloridate has now been successfully prepared by reaction of cholestanol with pyrophosphoryl chloride; the formation of the phosphorodichloridate was first indicated by the breakdown pattern on t.l.c. which was similar to that shown by cholesteryl phosphorodichloridate. Treatment of epicholestanol and epicholesterol in a similar manner gave the corresponding phosphorodichloridates (*cf.* ref. 2).

Epicholesteryl phosphorodichloridate (I) was more unstable (decomposed on t.l.c. using light petroleum), whereas the cholestanyl derivative only decomposed in ether–benzene; this is to be anticipated since 1,2-*trans*-diaxial elimination from (I) is particularly favoured as it leads to cholesta-3,5-diene (II):



In the phosphorylation of androst-5-en-3β-ol, Reiss [3] suggested that the intermediate phosphorodichloridate was solvolysed by pyridine, because t.l.c. appeared to indicate the presence of elimination products; however the validity of this evidence now seems doubtful in view of the decomposition of pure steroid phosphorodichloridates on t.l.c. plates. T.l.c. examination of the reaction between 3,5-cyclocholest-4-en-6β-ol and pyrophosphoryl chloride (in ether at −10°) indicated that on the introduction of the reagent there was rapid conversion into cholesterol, followed by normal phosphorylation so that the only isolable product was cholesteryl phosphorodichloridate (confirmed by preparation of the dimorpholidate derivative); presumably, the small amount of hydrogen chloride evolved causes the conversion of the cyclosteroid into cholesterol.[4] Treatment of 6β-hydroxycholest-3-one with pyrophosphoryl chloride gave cholestane-3,6-dione (confirmed by preparation of the mono-2,4-dinitrophenylhydrazone [5]); 1,3-diaxial interaction between the C(10)-angular methyl and the 6β-hydroxy groups inhibits phosphorylation and the starting material is isomerised to the 3,6-dione by trace amounts of hydrochloric acid (*cf.* ref. 6a).

Reactions of steroid phosphorodichloridates can occur *either* by replacement of the chlorine attached to the electrophilic phosphorus atom; *or* at the C(3)-carbon atom since the dichlorophosphoro-group is an effective leaving group. Cholesteryl, cholestanyl, ergosteryl, and lanosteryl phosphorodichloridates were treated with methanol. The cholestanyl and lanosteryl derivatives gave the corresponding dimethyl phosphates, while the cholesteryl (III) and ergosteryl (IV) derivatives reacted as shown in Scheme 1:



SCHEME 1

† Part I, R. J. W. Cremlyn, B. B. Dewhurst, and D. H. Wakeford, *J. Chem. Soc.* (*C*), 1971, 300.

[1] R. J. W. Cremlyn and N. A. Olsson, *J. Chem. Soc.*, 1969, 2305.
[2] D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 1951, 1048.
[3] J. Reiss, *Bull. Soc. chim. France*, 1965, 20.
[4] J. H. Beynon, I. M. Heilbron, and F. S. Spring, *J. Chem. Soc.*, 1936, 907.
[5] L. F. Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4377.
[6] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, (*a*) p. 204; (*b*) p. 316; (*c*) p. 314; (*d*) p. 33; (*e*) p. 16; (*f*) p. 253.

Similarly, by treatment with hot aqueous dioxan, cholestanyl and lanosteryl phosphorodichloridates [1] gave excellent yields of the corresponding dihydrogen phosphates; while the cholesteryl derivative gave a mixture of the dihydrogen phosphate (P-substitution) and some cholesteryl chloride (C-substitution); the ergosteryl derivative gave only 3,5-cycloergosta-6,8(14),22-triene (V). When cholesteryl phosphate was boiled with hydrochloric acid no cholesteryl chloride was formed, showing that the cholesteryl chloride does not arise *after* hydrolysis.

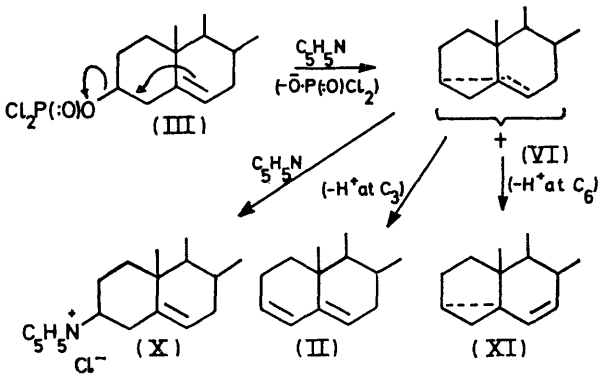
In the solvolysis of phosphorodichloridates like cholestanyl and lanosteryl phosphorodichloridates, substitution occurs entirely at the electrophilic phosphorus atom. However in compounds having a 5,6-double bond, anchimeric assistance may cause the C(3)-carbon atom to become an additional electrophilic centre; thus with these compounds two general reactions may be observed: (*a*) in the absence of base acid-catalysed loss of the dichlorophosphoro-group; and (*b*) in the presence of base P-substitution.

With ergosteryl phosphorodichloridate (IV), enhanced anchimeric assistance [7,8] causes ionisation of the dichlorophosphoro-group to become the dominant process giving the triene (V). Methanolysis in the *presence of pyridine*,[1] potassium acetate, or sodium ethoxide resulted in complete attack at phosphorus giving the dimethyl phosphate (VIII), indicating that the dichlorophosphoro-group is not such an effective leaving group as the toluene-*p*-sulphonyloxy [6b] (*cf.* ref. 9). The action of a tertiary base in facilitating replacement of the chlorine atoms of a phosphorodichloridate by alkoxy-groups is probably due to the base increasing the concentration of alkoxide ions, or possibly to the preliminary formation of a phosphorammonium salt (IX):

$$RO{\cdot}P(:O)Cl_2 + 2R'_3N \longrightarrow [(R'_3\overset{+}{N})_2P(:O){\cdot}OR]2Cl^-$$

Methanolysis of ergosteryl phosphorodichloridate in the presence of pyridine (2 mol.) still gave 3,5-cycloergosta-6,8(14),22-triene (V); with pyridine (8 mol.) a mixture of the triene and ergosteryl dimethyl phosphate was obtained; in the presence of pyridine (16 mol.) only the dimethyl phosphate was formed.

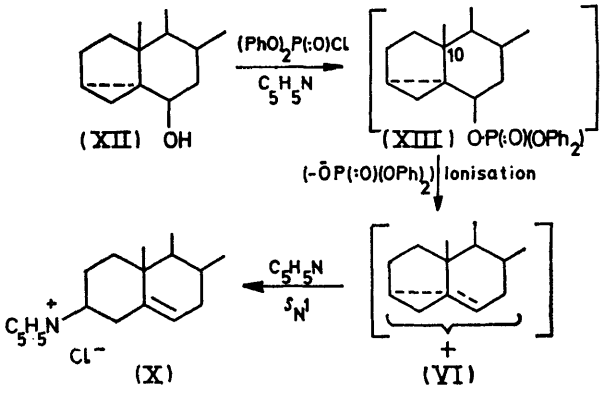
The reaction of cholesteryl phosphorodichloridate with a range of alcohols gave the corresponding cholesteryl ethers. Another illustration of the tendency of the dichlorophosphoro-group to function as a leaving group is provided by the solvolysis of steroid phosphorodichloridates with anhydrous pyridine. Cholesteryl, cholestanyl, and ergosteryl phosphorodichloridates gave the *N*-steroid pyridinium chlorides and hydrocarbons (*cf.* the pyridine solvolysis of steroid toluene-*p*-sulphonates); [11-13] for instance, cholesteryl phosphorodichloridate (III), gave a mixture of *N*-cholesterylpyridinium chloride (X), 3,5-cyclocholest-6-ene (XI), and some cholesta-3,5-diene (II):



The products probably arise from the homoallylic carbonium ion (VI) by simultaneous attack by pyridine at C(3) and loss of a proton from the C(3)- and C(6)-positions. The hydrocarbons also formed are probably a mixture of cholest-2- and -3-enes arising by a thermal (*cis*) elimination process.

With lanosteryl phosphorodichloridate only hydrocarbons were formed; presumably due to steric hindrance by the 4,4-dimethyl group preventing C(3)-substitution (S_N2) by pyridine.

Treatment of 3,5-cyclocholestan-6β-ol (XII) with diphenyl phosphorochloridate in pyridine gave *N*-cholesterylpyridinium chloride (X) [identical to the product (*cf.* ref. 14) from cholesteryl phosphorodichloridate–pyridine] which probably aroses from the intermediate diphenyl phosphate (XIII) as shown:



The isomerisation to the cholesteryl derivative must occur *after phosphorylation*, since otherwise the product would be cholesteryl diphenyl phosphate which is quite stable in the presence of pyridine under these conditions. In (XIII) there is 1,3-diaxial interaction

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[10] M. Ravel, J. Navech, and J. P. Vives, *Bull. Soc. chim. France*, *1963*, **10**, 2327.
[11] L. C. King and B. M. Regan, *J. Amer. Chem. Soc.*, 1952, **74**, 5617.
[12] L. C. King, R. M. Dodson, and L. A. Subluskey, *J. Amer. Chem. Soc.*, 1948, **70**, 1176.
[13] L. C. King and M. J. Bigelow, *J. Amer. Chem. Soc.*, 1952, **74**, 3338.
[14] J. H. Turnbull and W. Wilson, *J. Chem. Soc.*, 1954, 2301.

between the bulky diphenylphosphoro-group and the C(10)-angular methyl group, relieved by ionisation of the diphenylphosphoro-group forming the homoallylic carbonium ion (VI), and this gives *N*-cholesteryl-pyridinium chloride (X). Cholesteryl phosphorodichloridate (ref. 1) and cholesteryl dihydrogen phosphate can be hydrogenated to the cholestanyl derivatives; however efforts to hydrogenate dicholesteryl phosphorochloridate, dicholesteryl hydrogen phosphate, and tetracholesteryl pyrophosphate were unsuccessful.

EXPERIMENTAL

I.r. spectra were measured as Nujol mulls with an Infracord 237 spectrophotometer. N.m.r. spectra were determined with a Varian A60 spectrometer with tetramethylsilane as internal reference. U.v. spectra were measured with a Unicam SP 800 instrument. M.p.s were determined with a Kofler hot-stage apparatus. T.l.c. was carried out on silica gel G plates in benzene–ether (4 : 1); the plates were sprayed with 5% alcoholic dodecaphosphomolybdic acid and heated at 110° for 5 min. for development.

Cholestanyl Phosphorodichloridate.—Pyrophosphoryl chloride (2 g.) was added to cholestanol (1 g.) dissolved in the minimum volume of anhydrous ether. The mixture was left for 2½ hr. at room temperature when t.l.c. showed that no cholestanol remained. The solvent was removed under reduced pressure (temperature <30°) to give an oil which was extracted with light petroleum (b.p. 60—80°); the extract was washed with water, dried ($MgSO_4$), and evaporated (<30°) to give an oily residue (0·9 g.) (t.l.c. revealed the characteristic phosphorodichloridate decomposition pattern). Dissolution in anhydrous acetone and addition of methanol at −20° gave *cholestanyl phosphorodichloridate* as a white powder (500 mg.), m.p. 82—84° (Found: C, 64·0; H, 9·4; Cl, 13·9; P, 6·1. $C_{27}H_{47}Cl_2O_2P$ requires C, 64·2; H, 9·2; Cl, 14·0; P, 6·2%), $\nu_{max.}$ 1295 (P=O), 1010, 990 doublet cm.$^{-1}$ (P–O–C).

Epicholestanyl Phosphorodichloridate.—Epicholestanol (5α-cholestan-3α-ol) (2 g.) was treated with pyrophosphoryl chloride (4 g.) in ether, as previously described, to give *epicholestanyl phosphorodichloridate* (1·2 g.), m.p. 89° (Found: C, 64·5; H, 9·6; Cl, 13·9; P, 6·2. $C_{27}H_{47}Cl_2O_2P$ requires C, 64·2; H, 9·2; Cl, 14·0; P, 6·2%), $\nu_{max.}$ 1305 (P=O), 1020 cm.$^{-1}$ (P–O–C); $\nu_{max.}$ 970, 955, 740, and 640 cm.$^{-1}$ (these bands were absent in the spectrum of cholestanyl phosphorodichloridate).

Epicholesteryl Phosphorodichloridate.—Epicholesterol (cholest-5-ene-3α-ol) (0·9 g.), was treated with pyrophosphoryl chloride (1·0 g.) in ether. Partial evaporation of the ethereal solution, followed by addition of pentane, gave *epicholesteryl phosphorodichloridate* (530 mg.), m.p. 77° (Found: Cl, 13·5; P, 6·0. $C_{27}H_{45}Cl_2O_2P$ requires Cl, 14·1; P, 6·2%), $\nu_{max.}$ 1300 (P=O) and 1020 (P–O–C) cm.$^{-1}$; $\nu_{max.}$ 790, 720, 700, and 675 cm^{-1} (these bands were absent from the spectrum of cholesteryl phosphorodichloridate).

24-Methoxycarbonyl-5β-cholan-3-yl Phosphorodichloridate.—Methyl 3-hydroxy-5β-cholanate (3·6 g.) with pyrophosphoryl chloride (7·3 g.) in ether (90 ml.), gave the *phosphorodichloridate* as micro-needles (1·7 g.), m.p. 81—82° (Found: C, 59·3; H, 8·4; Cl, 13·4; P, 5·6. $C_{25}H_{41}Cl_2O_4P$ requires C, 59·3; H, 8·1; Cl, 13·9; P, 6·1%), $\nu_{max.}$ 1735 (C=O), 1300 (P=O), 1010, 980 cm.$^{-1}$ (P–O–C).

Phosphorylation of Ergost-8(14)-en-3β-ol.—A solution of ergost-8(14)-en-3β-ol (5 g.) in anhydrous pyridine (60 ml.) was treated with a solution of pyrophosphoryl chloride (8 ml.) in acetone,[1] to give the 3β-*phosphorodichloridate* as plates (4·2 g., 66%), m.p. 123—124° (Found: C, 54·2; H, 11·8; Cl, 17·6; P, 8·0. $C_{28}H_{47}Cl_2O_2P$ requires C, 54·4; H, 11·8; Cl, 17·9; P, 7·8%). $\nu_{max.}$ 1325 (P=O), 1040 (P–O–C) cm^{-1}.

T.l.c. of Cholesteryl Phosphorodichloridate.—Pure, recrystallised, cholesteryl phosphorodichloridate [1] dissolved in anhydrous chloroform was chromatographed on silica gel G, cellulose, and aluminium oxide plates. Each plate was developed with three different solvent systems: light petroleum (b.p. 30—40°), benzene–ether (4 : 1), and benzene–ether (1 : 4). With the first solvent the cholesteryl phosphorodichloridate never moved from the base line; with benzene–ether (4 : 1) and (1 : 4) mixtures on silica plates, it showed three spots of R_F 0·20, 0·42, and 0·76 (the major spot), and 0·58, 0·72, and 0·83 respectively; in each case there was considerable tailing between the spots. On the cellulose plates the compound always moved up to the solvent front, while on aluminium oxide it remained on the base line.

T.l.c. of the Reaction of Sterols with Pyrophosphoryl Chloride.—The characteristic decomposition of cholesteryl phosphorodichloridate with benzene–ether on silica plates, enables t.l.c. to be used to follow the phosphorylation of a sterol with pyrophosphoryl chloride: the sterol (100 mg.) was dissolved in the minimum volume of anhydrous ether, and a few drops of the solution was chromatographed. Pyrophosphoryl chloride (200 mg.) was added and the mixture was immediately chromatographed; further samples were taken after 0·5 1, 2, and 3 hr., the plates being developed with benzene–ether (4 : 1). A second silica plate developed with light petroleum (b.p. 30—40°) was also used to detect any hydrocarbons produced by dehydration. The results obtained with the following sterols were: cholesterol parent compound (R_F, 0·20), major product spot R_F 0·75; epicholestanol (R_F 0·33), R_F 0·75; epicholesterol (R_F 0·30), R_F 0·73; 3,5-cyclocholestan-6β-ol (R_F 0·39) on addition of the reagent a spot R_F 0·20 appears, then this is replaced by one of R_F 0·72; methyl 3α-hydroxy-5β-cholanate (R_F 0·15), R_F 0·60; 6β-hydroxycholest-4-en-3-one (R_F 0·09), R_F 0·33. The parent sterol spot disappeared after ½ hr. and was replaced by the major product spot which showed considerable tailing. In the plate developed with light petroleum, there was no movement from the base apart from the reactions with epicholestanol which gave a spot R_F 0·42, and epicholesterol (R_F 0·50).

Dicholesteryl Phosphoromorpholidate.—Dicholesteryl phosphorochloridate (10 g.) dissolved in hot benzene (150 ml.) was treated with a solution of morpholine (2·5 g.) in acetonitrile (60 ml.). The mixture was boiled under reflux for 4 hr. and the precipitated morpholine hydrochloride was filtered off; the filtrate was evaporated under reduced pressure. Recrystallisation of the residue from dioxan gave *dicholesteryl phosphoromorpholidate* as pale yellow plates (6·9 g.), m.p. 176° (Found: C, 76·7; H, 10·9; N, 1·7; P, 3·8. $C_{58}H_{98}NO_4P$ requires C, 77·1; H, 10·9; N, 1·55; P, 3·4%), $\nu_{max.}$ 1270 (P=O), 1260 (C–N), 1110 (C–O–C), 1020—980 (P–O–C) cm^{-1}.

Hydrolysis of Cholesteryl Phosphorodichloridate under Various Conditions.—(a) *With aqueous dioxan*. The phosphorodichloridate (5 g.) upon treatment with 5% aqueous dioxan (100 ml.) for 2 hr. at 55°, gave cholesteryl dihydrogen

phosphate as plates (3·6 g., 77%), m.p. 186—188° (lit.,[1] 186—188°); the filtrate gave cholesteryl chloride (0·17 g., 4%), m.p. 96° (lit.,[6d] 97°). When the phosphorodichloridate (5 g.) was boiled under reflux for 3 hr. with 10, 20, 30, and 40% aqueous dioxan the yields of cholesteryl dihydrogen phosphate and chloride were 31, 35, 38, 50, and 22, 20, 18, 18% respectively.

(b) *With aqueous dioxan–pyridine.* Cholesteryl phosphorodichloridate (10 g.) was boiled under reflux with 5% aqueous dioxan (200 ml.) containing pyridine (3·2 g., 1 mol.) for 3 hr. to give *cholesterylpyridinium phosphate monohydrate* from dioxan (6·7 g.), m.p. 166—170° (Found: C, 68·3; H, 9·3; N, 2·6; P, 5·1. $C_{27}H_{47}O_4P,C_5H_5N,H_2O$ requires C, 68·2; H, 9·6; N, 2·5; P, 5·5%), $\nu_{max.}$ 3480 (OH), 1300—900 cm.$^{-1}$ (a continuous diffuse absorption).

The product, after being boiled with glacial acetic acid–dioxan for 22 hr., gave *cholesteryl hemipyridinium phosphate hemihydrate*, m.p. 160—162° (Found: C, 68·7; H, 9·5; N, 1·6; P, 5·7. $C_{27}H_{47}O_4P,\frac{1}{2}C_5H_5N,\frac{1}{2}H_2O$ requires C, 68·7; H, 9·8; N, 1·4; P, 6·0%); the i.r. spectrum was unaltered. Equivalent weight (by potentiometric titration with tetrabutylammonium hydroxide in aqueous tetrahydrofuran) 259 (Calc. 257). Treatment of the pyridinium monohydrate with boiling ethanol–0·5N-sulphuric acid for 2½ hr. gave cholesteryl dihydrogen phosphate, m.p. 185°.

A similar experiment with aqueous dioxan–2,6-dimethylpyridine for hydrolysis gave *cholesteryl-2,6-dimethylpyridinium phosphate monohydrate*, m.p. 166—168° (Found: C, 68·75; H, 9·6; N, 2·3; P, 5·65. $C_{27}H_{47}O_4P,C_7H_9N,H_2O$ requires C, 69·0; H, 9·9; N, 2·4; P, 5·25%).

(c) *With dioxan–concentrated hydrochloric acid.* The phosphorodichloridate (1·5 g.) was boiled with dioxan (30 ml.) containing concentrated hydrochloric acid (1 g.) under reflux for ½ hr. to give cholesteryl chloride (0·9 g., 75%), m.p. 97° (lit.,[6d] 97°).

(d) *With aqueous tetrahydrofuran.* The phosphorodichloridate (5 g.) was heated with 5% aqueous tetrahydrofuran (100 ml.) at 55° for 2 hr. to give cholesteryl dihydrogen phosphate (59%).

Ergosteryl Dihydrogen Phosphate.—Attempted hydrolysis of ergosteryl phosphorodichloridate (5 g.) with boiling 5% aqueous dioxan (100 ml.) during 3 hr. was unsatisfactory and gave a mixture of products (R_F 0·45, 0·52, 0·62, 0·67, and 0·78). Use of 5% aqueous tetrahydrofuran at 55° for 2 hr. gave ergosteryl dihydrogen phosphate (1·2 g., 26%), m.p. 168° (lit.,[15] 165—168°), $\nu_{max.}$ 1260 (P=O), 1600 (conj. C=C), 1060 (P–O–C), and 2350 cm.$^{-1}$ (P–OH). T.l.c. of the filtrate showed the presence of 3,5-cycloergosta-6,8(14),22-triene, $\lambda_{max.}$ (cyclohexane) 261 mμ (ε 23,300), R_F 0·78.

Cholestanyl Dihydrogen Phosphate.—Cholestanyl phosphorodichloridate (5 g.) was boiled under reflux with 5% aqueous dioxan (100 ml.) for 3 hr. The cool reaction mixture gave *cholestanyl dihydrogen phosphate* as lustrous plates (3·4 g., 73%), m.p. 156° (Found: C, 69·5; H, 10·0; P, 6·2. $C_{27}H_{49}O_4P$ requires C, 69·5; H, 10·2; P, 6·6%), $\nu_{max.}$ 1270 (P=O), 1040 (P–O–C), 2350 (P–OH) cm^{-1}. T.l.c. of the filtrate gave no indication of cholestanyl chloride.

Reaction of Cholesteryl Phosphorodichloridate with Methanol under Various Conditions.—(a) *Methanol–acetonitrile.* The phosphorodichloridate (5 g.) was boiled under reflux with a 40% solution of methanol in anhydrous acetonitrile (100 ml.) for 20 min. to give cholesteryl methyl ether as needles from acetone (3·4 g., 87%), m.p. 83° (lit.,[5c] 84°), i.r. spectrum identical to that of an authentic sample. T.l.c. of the mother liquor showed two spots R_F 0·62 (cholesteryl methyl ether) and 0·77 (cholesta-3,5-diene); $\lambda_{max.}$ (2,2,4-trimethylpentane) 235 mμ (ε 3960) indicating ≃20% of the diene.[6f]

(b) *Methanol–concentrated sulphuric acid.* The phosphorodichloridate (5 g.) was heated under reflux with methanol (100 ml.) containing concentrated sulphuric acid (1 g.) to give cholesteryl methyl ether (1·2 g., 31%), m.p. 83°. The filtrate gave cholesteryl dimethyl phosphate as plates from light petroleum (b.p. 60—80°) (2·4 g., 50%), m.p. 125—126° (lit.,[1] 127—128°), mixed m.p. with an authentic sample 124—126°.

(c) *Methanol–potassium acetate.* The phosphorodichloridate (5 g.), was boiled under reflux with anhydrous potassium acetate (4·3 g.) in methanol (100 ml.) for 3 hr., and poured onto ice–water. Extraction with ether (2 × 100 ml.), concentration of the ethereal extract, and chromatography with aluminium oxide with ether as eluant gave cholesteryl dimethyl phosphate as plates from light petroleum (b.p. 60—80°) (3·9 g., 80%), m.p. 126—127°.

Cholestanyl Dimethyl Phosphate.—Cholestanyl phosphorodichloridate (5 g.) was boiled under reflux with methanol (100 ml.) for ½ hr. The solution was poured onto ice–water, extracted with ether (2 × 100 ml.), washed with water, and evaporated to give a gummy residue. Crystallisation from light petroleum (b.p. 60—80°) gave *cholestanyl dimethyl phosphate* (3·5 g., 72%), m.p. 116° (Found: C, 70·1; H, 10·4; P, 6·1. $C_{29}H_{53}O_4P$ requires C, 70·4; H, 10·5; P, 6·3%), $\nu_{max.}$ 1270 (P=O), 1190 (P–O–Me), 1040 cm.$^{-1}$ (P–O–C); τ ($CDCl_3$) showed a strong doublet at 6·25 (*J* 14 Hz). T.l.c. of the filtrate gave a single spot R_F 0·11 (cholestanyl dimethyl phosphate).

Reaction of Ergosteryl Phosphorodichloridate in the Presence of Different Amounts of Pyridine.—Ergosteryl phosphorodichloridate (1 g.) was boiled under reflux with methanol (100 ml.) containing pyridine (0·4 ml., 2·5 mole) for 3 hr. The cool mixture gave 3,5-cycloergosta-6,8(14),22-triene as needles from acetone (0·5 g.), m.p. 97°, $\lambda_{max.}$ 261 mμ (lit.,[1] 94—97°, 261 mμ).

A similar experiment in the presence of pyridine (9·5 mol.), gave the triene (153 mg., 21%), m.p. 98°, $\lambda_{max.}$ 261 mμ, and the filtrate, when cooled, gave ergosteryl dimethyl phosphate (392 mg., 40%), m.p. 153—154°, $\nu_{max.}$ 1270 (P=O), 1190 (P–O–Me), 1040 cm.$^{-1}$ (P–O–C). With more pyridine (16 mol.), the sole product isolated was ergosteryl dimethyl phosphate (600 mg., 61%), m.p. 152—153°.

Reaction of Cholesteryl Phosphorodichloridate with Other Alcohols.—(a) *Isopropyl alcohol.* The phosphorodichloridate (5 g.) was boiled under reflux with isopropyl alcohol (100 ml.) for 20 min. When cooled overnight the solution gave cholesteryl isopropyl ether as needles (2·3 g., 54%), m.p. 130—132° (lit.,[16] 132°) (Found: C, 84·3; H, 11·7. Calc. for $C_{30}H_{52}O$: C, 84·1; H, 12·1%), $\nu_{max.}$ 1080 cm.$^{-1}$ (C–O–C).

(b) *t-Butyl alcohol.* The phosphorodichloridate and t-butyl alcohol, under similar conditions, gave cholesteryl t-butyl ether as needles from ethyl acetate (2·5 g., 56%), m.p. 170—172° (lit.,[17] 172—173°) (Found: C, 83·6; H, 11·6. Calc. for $C_{31}H_{54}O$: C, 84·1; H, 12·2%).

(c) *Cyclohexanol.* The phosphorodichloridate (5 g.) was boiled under reflux with cyclohexanol (10 g.) in acetonitrile (90 ml.) for 1 hr. The mixture was cooled to give choles-

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teryl cyclohexyl ether as needles from light petroleum (b.p. 60—80°) (3·0 g., 64%), m.p. 160—161° (lit.,[18] 153—156°) (Found: C, 84·7; H, 12·1. Calc. for $C_{33}H_{57}O$: C, 84·4; H, 12·15%), $\nu_{max.}$ 1080 cm.$^{-1}$ (C–O–C).

(d) *Borneol.* Under similar conditions to those employed in (c), bornyl cholesteryl ether was obtained as lustrous plates from benzene–methanol (1·6 g., 30%), m.p. 172° (lit.,[19] 177°) (Found: C, 85·0; H, 12·1. Calc. for $C_{37}H_{63}O$: C, 84·9; H, 12·25%), $\nu_{max.}$ doublet at 1120, 1100 cm.$^{-1}$ (C–O–C).

(e) 1-*Methylcyclohexanol.* Cholesteryl phosphorodichloridate (3·8 g.) in nitromethane (60 ml.) was heated to the b.p. with 1-methylcyclohexanol (5·3 g.) and then immediately cooled to give *cholesteryl 1-methylcyclohexyl ether* (0·9 g.), m.p. 195—197° after recrystallisation from acetone–carbon tetrachloride (Found: C, 84·2; H, 12·0. $C_{34}H_{59}O$ requires C, 84·5; H, 12·2%). In a similar experiment in acetonitrile no cholesteryl 1-methylcyclohexyl ether was isolated.

(f) *Benzyl alcohol.* The phosphorodichloridate (5 g.) was heated with benzyl alcohol (40 ml.) until all the solid had dissolved. Solid separated from the cooled mixture and this was recrystallised from ethyl acetate to give benzyl cholesteryl ether as needles (2·4 g., 52%), m.p. 114—115° (lit.,[20] 116°), $\nu_{max.}$ 3060, 3040, 3020 (Ar C–H), 1110 (C–O–C), 740, 705 cm.$^{-1}$ (mono. subst. benzene).

Reaction of Cholesteryl Phosphorodichloridate with Pyridine.—Method 1. The phosphorodichloridate (5 g.) was boiled under reflux with anhydrous pyridine (50 ml.) for 3 hr. to give a crystalline precipitate. Recrystallisation of this from aqueous acetone gave plates (1·3 g.), m.p. 310° (decomp.). The compound contained ionic chlorine and no phosphorus. The i.r. spectrum showed a strong broad band at 3400 and sharp intense bands at 1620 and 680 cm.$^{-1}$. Treatment with warm aqueous methanol–ammonium nitrate solution gave a precipitate; this was recrystallised from ethanol to give a white powder, m.p. 350°. Evaporation of the original filtrate gave a solid (1·2 g.), m.p. 65—80°, t.l.c. (light petroleum b.p. 30—40°) showed two spots R_F 0·37 and 0·50, $\lambda_{max.}$ (2,2,4-trimethylpentane) 235 (ε 1553) and at 215 mμ (6860) suggesting *ca.* 80% of 3,5-cyclocholest-6-ene (*cf.* ref. 21) and 8% of cholesta-3,5-diene [6e] (ε 20,000).

Method 2. The phosphorodichloridate (2·2 g.) dissolved in the minimum volume of anhydrous ether was treated with pyridine (2 ml.) at 0°. The solution was left overnight at 0°, and concentrated to give a solid (1·3 g.), m.p. 110°, $\nu_{max.}$ 1300 (P=O), 1020 (P–O–C), also a diffuse band at 3400 and a doublet at 1640 and 1540 cm^{-1}.

Reaction of Pyridine with Other Steroid Phosphorodichloridates.—(a) *Cholestanyl phosphorodichloridate.* The phosphorodichloridate (5 g.) was boiled with anhydrous pyridine (50 ml.) for 3 hr. to give plates (0·9 g.), m.p. 296—298°. The i.r. spectrum was similar to that of *N*-cholesterylpyridinium chloride. Treatment with warm methanol–ammonium nitrate gave a white powder from ethanol, decomp. p. 315—320°.

The original filtrate gave a white solid from acetone (1·3 g.), m.p. 67—75°. T.l.c. showed two spots R_F 0·45, 0·65, probably a mixture of cholest-2- and -3-enes (ref. 6*f*) (Found: C, 87·7; H, 11·8. Calc. for $C_{27}H_{44}$: C, 88·0; H, 12·0%).

(b) *Ergosteryl phosphorodichloridate.* The phosphorodichloridate (5 g.) was treated with pyridine (50 ml.), as previously described, to give pale yellow crystals (600 mg.), m.p. 244° (decomp.) (i.r. spectrum similar to that of *N*-cholesterylpyridinium chloride). Treatment with methanol–ammonium nitrate gave lustrous plates from ethanol, m.p. 277—278° (decomp.).

The original filtrate when cooled gave pure 3,5-cycloergosta-6,8(14),22-triene as needles from acetone (827 mg.), m.p. 101° $\lambda_{max.}$ (2,2,4-trimethylpentane) 261 mμ (ε 26,800). Evaporation of solvent gave an oily solid (1·3 g.), m.p. 97° (after crystallisation from acetone). In the u.v. spectrum the main band is at 261 mμ [the 6,8(14),22-triene], but there are other bands at 292, 315, and 331 mμ (Ergosta-3,5,7,22-tetraene has a band at 316 mμ [22]).

(c) *Lanosteryl phosphorodichloridate.* The phosphorodichloridate, (5 g.) on treatment with pyridine, gave no *N*-lanosterylpyridinium chloride, but only a solid from aqueous ethanol (2·5 g.), m.p. 58—64° (No Cl, N, P). T.l.c. (light petroleum b.p. <40°) showed two hydrocarbons to be present R_F 0·51, 0·63 (Found: C, 88·1; H, 11·8. Lanostene, $C_{30}H_{48}$, requires C, 88·3; H, 11·7%).

Hydrogenation of Cholesteryl Dihydrogen Phosphate.—Cholesteryl dihydrogen phosphate (3 g.) in dry tetrahydrofuran (20 ml.) containing perchloric acid (2 drops of 60%) and Adams catalyst (90 mg.) was hydrogenated at atmospheric pressure [calculated volume of hydrogen (175 ml.) was absorbed in 1½ hr.]. The catalyst was removed and the tetrahydrofuran was evaporated off under reduced pressure. The residue was crystallised from dioxan to give cholestanyl dihydrogen phosphate (2·4 g., 80%), m.p. 156° (identical i.r. and m.p. to the authentic compound from hydrolysis of cholestanyl phosphorodichloridate).

Treatment of Cholesteryl Dihydrogen Phosphate with Hydrochloric Acid.—Cholesteryl dihydrogen phosphate (1 g.) was boiled under reflux with concentrated hydrochloric acid (0·5 ml.) in dioxan (25 ml.) for 3 hr. Only unchanged material (0·8 g.), m.p. 186° was obtained, and there was no indication of cholesteryl chloride formation.

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