Some Steroid Phosphates and Related Compounds

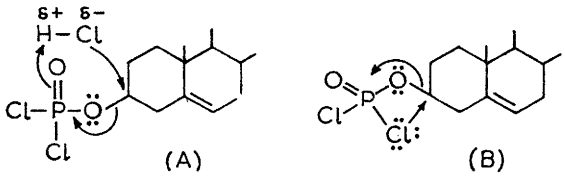
By **R. J. W. Cremlyn** * and **N. A. Olsson**, Department of Chemical Sciences, The Hatfield Polytechnic, Hatfield, Hertfordshire

The preparation of cholesteryl dihydrogen phosphate *via* cholesteryl phosphorodichloridate is described; although the reaction was successful for the preparation of ergosteryl and lanosteryl phosphorodichloridates, it failed with cholestanol and thiocholesterol. Dicholesteryl phosphorochloridate has been prepared but not diergosteryl or dilanosteryl phosphorochloridates. The hydrolysis of cholesteryl phosphorodichloridate has been examined. Reaction of thiophosphoryl chloride and cholesterol gave cholesteryl thionophosphorodichloridate but this could not be hydrolysed to the phosphate. Treatment of cholesterol with phosphorus pentasulphide gave *OO*-dicholesteryl hydrogen phosphorodithioate contrary to previous reports. A study has been made of the decomposition of cholesteryl phosphorodichloridate in inert organic solvents.

CHOLESTERYL dihydrogen phosphate is reported [1] to have been made by the oxidation of cholesteryl dihydrogen phosphite, and by treatment of cholesterol with phosphorus oxychloride.[2-7] The recorded melting points are 148° (ref. 1) (this is probably unchanged cholesterol), and 170—196°.[2] By the latter route we obtained products with melting points in the range 180—195°; the action of pyrophosphoryl chloride [8] on cholesterol however gave cholesteryl dihydrogen phosphate which melted at 186—188°. Since no pyridine is used in this procedure it is possible that the variation in the previous melting points arises from traces of pyridine in the product rather than from differing degrees of hydration. In this connection, it has been observed [9] that the melting points of steroid phosphates do not offer the normal criteria of purity since they are liable to vary in different preparations. Venner [7] found that ethanol was the most satisfactory solvent for the crystallisation of cholesteryl dihydrogen phosphate, Plimmer and Burch [4] chloroform for the recrystallisation whilst we found dioxan to be the best solvent. Treatment of cholesterol in acetone–pyridine with phosphorus oxychloride [7] gave cholesteryl phosphochloridate in 92% yield.

This method has been extended to the preparation of ergosteryl (63%) and lanosteryl phosphorodichloridates (49%); it failed however with cholestanol and thiocholesterol. By altering the order of addition, dicholesteryl phosphorochloridate (71%) was also obtained although attempts to prepare diergosteryl and dilanosteryl phosphorochloridates failed. Tricholesteryl phosphate was obtained by the action of cholesterol on dicholesteryl phosphorochloridate. The steroid phosphorodichloridates melted to red liquids. Although cholesteryl and lanosteryl phosphorodichloridates were reasonably stable in stoppered vessels cholestanyl and ergosteryl phosphorodichloridates slowly evolved hydrogen chloride and the white solids became respectively red (24 hr.) or green (48 hr.); when dissolved in chloroform the latter compounds decomposed to give black solutions (3 days). Treatment of cholesteryl phosphorodichloridate with morpholine in light petroleum or acetonitrile gave, respectively a mono- or a di-morpholine salt.

Actually the attempted phosphorylation of cholesterol with dibenzyl phosphorochloridate [10] gave only starting material (*cf.* ref. 7.) Treatment with pyrophosphoryl chloride [11] afforded an excellent yield of cholesteryl phosphorodichloridate. This result contrasts with the unsuccessful attempts of Slates *et al.*[11] to phosphorylate C_{21}-hydroxy-steroids with the same reagent. A further difference is that cholesteryl phosphorodichloridate has a solubility in ice–water and is, consequently, only hydrolysed slowly. The hydrolysis of cholesteryl phosphorodichloridate was studied in order to improve the reported low yield (16·5%) of cholesteryl dihydrogen phosphate.[7] Treatment of the compound with boiling water afforded a 65% yield of cholesteryl dihydrogen phosphate; with boiling aqueous dioxan the yield of the latter compound was 46% with 12% of cholesteryl chloride; boiling aqueous acetone gave 35% cholesteryl dihydrogen phosphate and 6% of cholesteryl chloride whilst boiling aqueous triethylamine and aqueous pyridine gave P_1,P_2-dicholesteryl pyrophosphate. Boiling aqueous lithium hydroxide gave a 50% yield of dilithium cholesteryl phosphate.



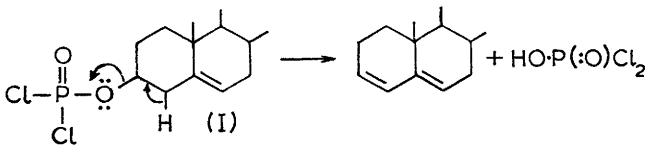
SCHEME 1

The cholesteryl chloride formed in these hydrolyses probably arises, in the main, from intermolecular S_N2 attack by the evolved hydrogen chloride [See (A) in Scheme 1] rather than by S_Ni type rearrangement of the phosphorodichloridate [see (B) in Scheme 1] since only low yields of cholesteryl chloride were obtained the

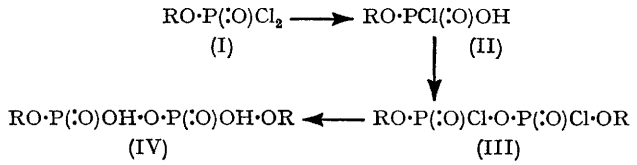
[1] N. Takashima, *J. Pharm. Soc. Japan*, 1928, **48**, 878.
[2] H. von Euler and A. Bernton, *Ber.*, 1927, **60**, 1720.
[3] H. von Euler, A. Wolf, and H. Hellström, *Ber.*, 1929, **62**, 2451.
[4] R. H. A. Plimmer and W. J. N. Burch, *J. Chem. Soc.*, 1929, 279, 292.
[5] J. H. Turnbull and W. Wilson, *J. Chem. Soc.*, 1954, 2301.
[6] H. A. C. Montgomery and J. H. Turnbull, *J. Chem. Soc.*, 1956, 4606.
[7] H. Venner, *J. prakt. Chem.*, 1960, **12**, 59.
[8] H. Grunze and W. Koransky, *Angew. Chem.*, 1959, **71**, 407.
[9] J. Reiss, *Bull. Soc. chim. France*, 1965, 18.
[10] G. W. Kenner, A. R. Todd, and F. J. Weymouth, *J. Chem. Soc.*, 1952, 3675.
[11] H. L. Slates, S. Weber, and N. L. Wendler, *Chem. and Ind.*, 1967, 1174.

compound was decomposed in anhydrous solvents, and none when it was carried out in the presence of bases.

When cholesteryl phosphorodichloridate was warmed in dioxan the main product was cholesta-3,5-diene, an indication that the dichlorophosphoro-group is readily eliminated:



On treatment of cholesteryl phosphorodichloridate (I) with aqueous pyridine, dissolution of the phosphorodichloridate was incomplete, in contrast to an analogous experiment with aqueous triethylamine; apparently heterogeneous conditions favour the formation of P_1P_2-dicholesteryl pyrophosphate (IV). This product is probably obtained *via* the hydroxy-chloridate (II) and the intermediate dicholesteryl pyrophosphoryl chloride (III) (see Scheme 2):



R = Cholesteryl.

SCHEME 2

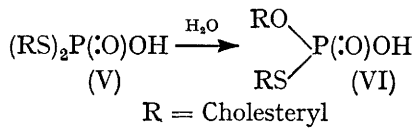
Other mechanisms involving condensation of cholesteryl dihydrogen phosphate or two molecules of the hydroxy-chloridate (II) are less likely since the introduction of the hydroxy-group should reduce the reactivity of the chlorine atom.

Direct phosphorylation of cholestanol failed to give cholestanyl phosphorodichloridate and the compound was prepared indirectly by hydrogenation of cholesteryl phosphorodichloridate; it was characterised by conversion into the phosphate and the dimorpholine salt. Treatment of cholesterol, cholestanol, and ergosterol with diphenyl phosphorochloridate gave the corresponding diphenyl phosphates.

Although phosphorus pentasulphide normally reacts with an alcohol to form the corresponding *OO*-dialkyl hydrogen phosphorodithioate,[12] Montignie [13] reported that with cholesterol thiocholesterol was obtained;

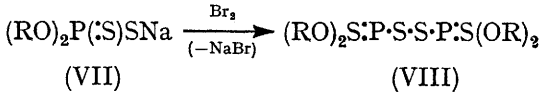
$$4ROH + P_2S_5 \longrightarrow 2(RO)_2PS{\cdot}SH + H_2S$$

in contrast Wagner–Jauregg *et al.*[14] claimed that *SS*-dicholesterylphosphorodithioate (V) was formed, which upon treatment with boiling water gave *O*-cholesteryl *S*-cholesterylphosphorothioate (VI):



R = Cholesteryl

We repeated the latter reaction but obtained only the normal product (VII) which was unchanged upon being boiled with water. Evidence in support of the suggested identity for the product was as follows. Treatment of an alcoholic solution of the sodium salt with bromine gave the disulphide (VIII) (*cf.* ref. 15):

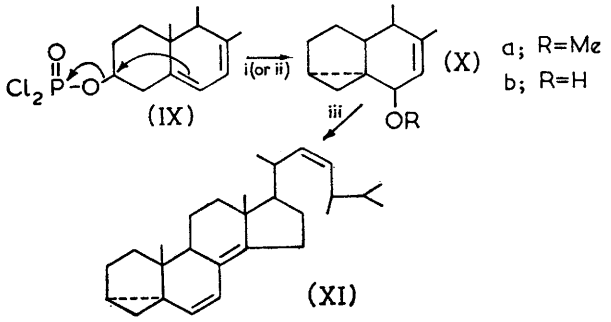


Methylation of the sodium salt gave *OO*-dicholesteryl *S*-methyldithioate which indicated that compound (VII) contained a P–SH group (see Experimental section). The i.r. spectrum of (VII) showed no absorption for P=O [16a] but strong absorption in the P–O–C region (1050—950 cm.$^{-1}$),[16b] and a sharp band in the P=S region 750—600 cm.$^{-1}$.[16c] Cholesterol with thiophosphoryl chloride in acetone gave cholesteryl thionophosphorodichloridate, which was characterised by preparation of the mono- and di-morpholine salts. Attempts to hydrolyse cholesteryl thionophosphorodichloridate including treatment with ethanolic potassium hydroxide (*cf.* ref. 17) to the corresponding phosphate were unsuccessful. The reaction was complex and involved loss of hydrogen sulphide, replacement of the dichlorothiophosphoro-group, and partial conversion of the thiophosphoryl into the phosphoryl group. In contrast to the preceding reaction, the action of sodium ethoxide on cholesteryl thionophosphorodichloridate afforded the thionodiethyl phosphate. Cholesteryl phosphorodichloridate with hot methanol, under basic conditions, gave cholesteryl dimethyl phosphate; on the other hand under acid conditions, a mixture of the dimethyl phosphate and cholesteryl methyl ether was formed. The formation of the latter compound is possibly due to acid conditions which cause greater electron withdrawal from the C-3-position of the steroid nucleus, thus facilitating attack by methanol at this position as well as at the electrophilic phosphorus atom.

When ergosteryl phosphorodichloridate (IX) is heated with methanol, the product is 3,5-cycloergosta-6,8-(14),22-triene (XI); this compound was also obtained, together with ergosteryl phosphorodichloridate, by the action of pyrophosphoryl chloride on ergosterol. These results suggest that ergosteryl phosphorodichloridate is

[12] G. M. Kosolapoff, 'Organophosphorus Compounds,' John Wiley, New York, 1950, p. 236.
[13] E. Montignie, *Bull. Soc. chim. France*, 1931, **49**, 73.
[14] T. Wagner-Jauregg, T. Lennartz, and H. Kothny, *Ber.*, 1941, **74**, 1513; T. Wagner-Jauregg and T. Lennartz, *ibid.*, 1942, **75**, 178.
[15] H. U. Ping-Fang and Cheng Wan-yi, *Acta Chim. Sinica.*, 1956, **22**, 215.
[16] L. J. Bellamy, 'The Infra-Red Spectra of Complex Molecules,' Methuen, 2nd edn., 1958, (*a*) p. 313; (*b*) p. 316; (*c*) p. 321; (*d*) p. 323; (*e*) p. 258; (*f*) p. 116; (*g*) p. 40; (*h*) p. 319; (*i*) p. 18.
[17] T. W. Mastin, G. R. Norman, and T. A. Weilmuenster, *J. Amer. Chem. Soc.*, 1945, **67**, 1662.

probably the intermediate in the formation of 3,5-cycloergosta-6,8(14),22-triene by the action of phosphorus oxychloride on ergosterol,[18c] as indicated below:



Reagents: i, MeOH; ii, aq. Na_2CO_3; iii, $^-$ROH.

The cyclosteroid rearrangement also occurred when ergosteryl diphenyl phosphate was heated with sodium hydrogen carbonate–acetone to give (Xb). However the action of sodium methoxide on ergosteryl phosphorodichloridate gave ergosteryl dimethyl phosphate.

In contrast Venner [7] suggested that the major product from the action of phosphorus oxychloride on ergosterol was diergosteryl ether, we have shown, however, that the triene (XI) is formed. Venner [7] obtained only 2% of ergosteryl dihydrogen phosphate, as compared with 80% of lumisteryl dihydrogen phosphate from the analogous reaction of lumisterol with phosphorus oxychloride; the much higher yield from lumisterol is probably due to inversion of the C(10)-angular methyl group in this compound which is known [18d] to inhibit cyclosteroid formation.

EXPERIMENTAL

I.r. spectra were measured as Nujol mulls with an Infracord 237 spectrometer and far i.r. spectra with a Grubb–Parsons D.M.4 spectrophotometer.

N.m.r. spectra were determined with a Varian A60 spectrophotometer with tetramethylsilane as internal standard.

Cholesteryl Phosphorodichloridate.—Method (a); *with phosphorus oxychloride.* Cholesterol (20 g.) was treated with phosphorus oxychloride in acetone–pyridine as described by Venner.[7] Crystallisation of the product from light petroleum (b.p. 60—80°) gave cholesteryl phosphorodichloridate as needles (19·7 g., 92%), m.p. 122° (lit.,[3] 122°) (Found: Cl, 13·6. Calc. for $C_{27}H_{45}Cl_2O_2P$: Cl, 14·1%), $\nu_{max.}$ 1300 (P=O), 536 and 429 (P–Cl),[16d] 1020 (P–O–C) cm.$^{-1}$.

Method (b); *with pyrophosphoryl chloride.* Cholesterol (10 g.) dissolved in the minimum volume of anhydrous ether was added to pyrophosphoryl chloride (17 c.c.) at 0°. The crystalline precipitate was filtered off after ½ hr. and washed with acetone and ice-cold ether to give cholesteryl phosphorodichloridate (11·3 g., 85%), m.p. 117—119°. The use of tetrahydrofuran as solvent gave a lower yield (40%) of cholesteryl phosphorodichloridate.

Cholesteryl Morpholinophosphorochloridate.—To cholesteryl phosphorodichloridate (5 g.) dissolved in hot, light petroleum (b.p. 60—80°) (50 c.c.) was added morpholine (1·7 g.), the mixture was heated under reflux for 4 hr. The solvent was removed under reduced pressure, and the residue was crystallised from ethanol to give the *morpholinophosphorochloridate* as prisms (2·3 g.), m.p. 147° (Found: C, 67·2; H, 9·55; Cl, 6·0; N, 2·4; P, 5·1. $C_{31}H_{54}ClNO_3P$ requires C, 67·1; H, 9·6; Cl, 6·4; N, 2·5; P, 5·6%), $\nu_{max.}$ 1280 (P=O), 1260 (C–N),[16e] 1120 (C–O–C),[16f] 1030 (P–O–C), 538 and 427 (P–Cl) cm.$^{-1}$.

Cholesteryl Phosphorodimorpholidate.—Cholesteryl phosphorodichloridate (5 g.) and morpholine (10 c.c.) in acetonitrile (250 c.c.) was boiled for ¼ hr. and then cooled. The *phosphorodimorpholidate* crystallised as needles (3·3 g.), m.p. 159—160° (Found: C, 69·4; H, 10·1; N, 4·5; P, 5·0. $C_{35}H_{61}N_2O_4P$ requires C, 69·5; H, 10·1; N, 4·6; P, 5·1%), $\nu_{max.}$ 1210 (P=O), 1260 (C–N), 1120 (C–O–C), 1040 (P–O–C) cm.$^{-1}$.

Ergosteryl Phosphorodichloridate.—Method (a); *with Phosphorus oxychloride.* A solution of ergosterol (5 g.) in anhydrous chloroform–pyridine (60 c.c., 1 : 7·5) was added dropwise during 5 hr. to a solution of phosphorus oxychloride (4·6 c.c.) in anhydrous acetone (60 c.c.) at 0°. The precipitate crystallised from light petroleum to give *ergosteryl phosphorodichloridate* as needles (4·0 g., 63%), m.p. 84° (decomp.) (Found: Cl, 13·4; P, 5·6. $C_{28}H_{43}Cl_2O_2P$ requires Cl, 13·8; P, 6·0%), $\nu_{max.}$ 1605 (conj. C=C),[16g] 1320 (P=O), 1050 (P–O–C) cm.$^{-1}$.

Method (b); *with pyrophosphoryl chloride.* Ergosterol (10 g.) was treated with the reagent (12 c.c.), as previously described for cholesterol to give ergosteryl phosphorodichloridate (3·8 g., 31%), m.p. 76°. The ethereal filtrate was treated with aqueous sodium hydrogen carbonate overnight; the ether layer was evaporated to give an orange solid which crystallised from acetone to give 3,5-cycloergosta-6,8(14),22-triene (1·0 g.), m.p. 102° (lit.,[19] 102°), $\lambda_{max.}$ (cyclohexane) 260 mμ, ε 24,000.

Ergosteryl Phosphorodimorpholidate.—Ergosteryl phosphorodichloridate (50 mg.) was heated with morpholine (0·5 c.c.) in acetonitrile (12 c.c.) for ¼ hr. to give *the phosphorodimorpholidate* (40 mg.), m.p. 145—147° (Found: C, 70·9; H, 9·6; N, 4·6, P, 5·3. $C_{36}H_{59}N_2O_4P$ requires C, 70·4; H, 9·6; N, 4·6; P, 5·1%).

Attempted Phosphorylation of Ergosterol.—Ergosterol (5 g.) was treated with phosphorous oxychloride as previously described, and was then set aside overnight at room temperature. The precipitate was crystallised from ethanol to give 3,5-cycloergosta-6,8(14),22-triene as plates (2 g.), m.p. 93°.

Cholestanyl Phosphorodichloridate.—Cholesteryl phosphorodichloridate (4·5 g.) in tetrahydrofuran (50 c.c.) containing Adams catalyst (200 mg.) was hydrogenated at atmospheric pressure [calculated vol. of H_2 (200 c.c.) was absorbed in 1½ hr.]. The catalyst was removed and the tetrahydrofuran was evaporated off under reduced pressure. The residue was crystallised from light petroleum (b.p. 60—80°) to give *cholestanyl phosphorodichloridate* (3·1 g.), m.p. 110—112° (Found: Cl, 13·7. $C_{27}H_{47}Cl_2O_2P$ requires: Cl, 14·0%). The compound gave a negative test with tetranitromethane in chloroform and was characterised as the dimorpholine salt.

[18] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, (*a*) p. 33; (*b*) p. 263; (*c*) p. 318; (*d*) p. 321; (*e*) p. 314; (*f*) p. 28.

[19] L. F. Fieser, M. Fieser, and M. Rosen, *J. Amer. Chem. Soc.*, 1952, **74**, 5397.

Cholestanyl Phosphorodimorpholidate.—Cholestanyl phosphorodichloridate (100 mg.) was warmed with morpholine (0·2 c.c.) in acetonitrile (3 c.c.); the mixture was cooled to give the *dimorpholidate* as needles (80 mg.), m.p. 152° (Found: C, 69·0; H, 10·1; N, 5·0; P, 4·7. $C_{35}H_{63}N_2O_4P$ requires C, 69·5; H, 10·1; N, 4·6; P, 5·1%).

Attempted Phosphorylation of Cholestanol.—(a) *With phosphorus oxychloride*. Treatment with phosphorus oxychloride in pyridine acetone for 4 hr. at 0° gave an oil; Attempts to characterise this as the dimorpholidate were unsuccessful (*cf.* ref. 9).

With (b) *Pyrophosphoryl chloride*. Treatment with this reagent (1 hr. at 0°) also gave an uncharacterised oil.

Cholesteryl Thionophosphorodichloridate.— Cholesterol (5 g.) in pyridine (25 c.c.) was added dropwise during 3 hr. to thiophosphoryl chloride (4·4 c.c.) in acetone (25 c.c.) at 0°. The precipitate was crystallised from light petroleum (b.p. 60—80°) to give *cholesteryl thionophosphorodichloridate* (4·0 g., 60%), m.p. 145° (decomp.) (Found: Cl, 13·8; P, 6·0; S, 6·3. $C_{27}H_{45}Cl_2OPS$ requires Cl, 13·7; P, 6·0; S, 6·2%), $\nu_{max.}$ 1020 (P–O–C), 740, 710 (P=S),[16c] 537 and 428, (P–Cl) cm.$^{-1}$.[16d]

Cholesteryl Morpholinothionophosphorochloridate.—Treatment of cholesteryl thionophosphorodichloridate (1 g.) with morpholine (as in the preparation of cholesteryl morpholinophosphorochloridate) gave the *morpholinothionophosphorochloridate* as a white powder (0·5 g.), m.p. 169—171° (from chloroform) (Found: Cl, 6·65; N, 2·3; P, 5·6; S, 6·3. $C_{31}H_{53}ClNO_2PS$ requires Cl, 6·7; N, 2·6; P, 5·7; S, 5·9%), $\nu_{max.}$ 1260 (C–N), 1120 (C–O–C), 1030 (P–O–C), 740, 670 (P=S) cm.$^{-1}$.

Cholesteryl Thionophosphorodimorpholidate.— Treatment of cholesteryl thionophosphorodichloridate (1 g.) with morpholine (as in the preparation of cholesteryl phosphorodimorpholidate) gave the *thionophosphorodimorpholidate* as needles (0·7 g.), m.p. 187° (from ethyl acetate) (Found: C, 67·5; H, 10·0; N, 4·5; P, 5·4; S, 5·6%. $C_{35}H_{61}N_2O_3PS$ requires C, 67·7; H, 9·8; N, 4·5; P, 5·0; S, 5·2%), $\nu_{max.}$ 1260 (C–N), 1120 (C–O–C), 750 (P=S) cm.$^{-1}$.

Dicholesteryl Phosphorochloridate.—*Method* (a). A solution of phosphorus oxychloride (8 c.c.) in acetone (100 c.c.) was added dropwise to a stirred solution of cholesterol (20 g.) in pyridine (100 c.c.) at 0° and the mixture was stirred overnight to give dicholesteryl phosphorochloridate as a white powder (15·1 g., 71%), m.p. 172° (lit.,[2] 172°) (Found: C, 76·6; H, 10·8; Cl, 4·5. Calc. for $C_{54}H_{90}ClO_3P$: C, 76·9; H, 10·7; Cl, 4·2%), $\nu_{max.}$ 1295 (P=O), 1030 (P–O–C) cm.$^{-1}$.

Method (b). Cholesteryl phosphorodichloridate (3 g.) and cholesterol (2·3 g.) dissolved in pyridine (30 c.c.) at 0°, were set aside for 5 hr. Crystalline dicholesteryl phosphorochloridate (3·9 g., 77%), m.p. 172°, was precipitated.

Lanosteryl Phosphorodichloridate.—Lanosterol (5 g.) in pyridine (25 c.c.) was added dropwise during 4 hr. to a stirred solution of phosphorus oxychloride (5 c.c.) in acetone (25 c.c.) at 0°, to give the *phosphorodichloridate* as a cream powder (3·1 g., 49%), m.p. 113° (decomp.) (Found: C, 66·1; H, 9·0; Cl, 13·0; P, 5·5. $C_{30}H_{49}Cl_2O_2P$ requires C, 66·3; H, 9·0; Cl, 12·9; P, 5·7%). $\nu_{max.}$ 1300 (P=O), 1010 (P–O–C) cm.$^{-1}$.

Lanosteryl Morpholinophosphorochloridate.— Lanosteryl phosphorodichloridate (500 mg.) in benzene (20 c.c.) was boiled under reflux with morpholine (0·2 c.c.) and pyridine (0·5 c.c.) for 5½ hr., to give the *morpholinophosphorochloridate* as plates (200 mg.) (from ethanol), m.p. 120—122° (Found: C, 68·0; H, 9·9; Cl, 5·6; N, 2·4; P, 5·0. $C_{34}H_{57}ClNO_3P$ requires C, 68·4; H, 10·1; Cl, 5·9; N, 2·3; P, 5·2%), $\nu_{max.}$ 1285 (PO), 1260 (C–N), 1120 (C–O–C), 1050 (P–O–C) cm.$^{-1}$.

Lanosteryl Phosphorodimorpholidate (with Mr. D. H. Wakeford).—Lanosteryl phosphorodichloridate (500 mg.) treated with morpholine, as previously described gave the *phosphorodimorpholidate* as needles (350 mg.), m.p. 159° (from ethanol) (Found: C, 70·4; H, 10·2; N, 4·2; P, 4·8. $C_{38}H_{65}N_2O_4P$ requires C, 70·8; H, 10·2; N, 4·35; P, 4·8%).

Attempted Preparation of Dilanosteryl Phosphorochloridate (with Mr. D. H. Wakeford).—Lanosterol with phosphorus oxychloride in acetone–pyridine (as described in the preparation of dicholesteryl phosphorochloridate) gave *lanosteryl phosphorodichloridate* m.p. 113—114° (decomp.) (Found: Cl, 12·7; P, 5·5. $C_{30}H_{49}Cl_2O_2P$ requires Cl, 12·9; P, 5·7%).

Attempted Preparation of Diergosteryl Phosphorochloridate. —*Method* (*a*). With phosphorus oxychloride in acetone–pyridine, a solid product (2·9 g.), m.p. 148°, was obtained. The i.r. spectrum indicated a free hydroxy-group (3300—3400), and absence of P=O and P–O–C groups.

Treatment of this product with an excess of morpholine in boiling chloroform gave plates, m.p. 200° (Found: C, 74·5; H, 9·4; N, 2·6; P, 3·2. $C_{60}H_{94}NO_4P$ requires C, 78·0; H, 10·2; N, 1·5; P, 3·4%).

Method (*b*). Treatment of ergosteryl phosphorodichloridate (2 g.) with ergosterol (1·5 g.) in pyridine (20 c.c.) at room temperature for 5 hr. gave a waxy solid which contained no chlorine. The i.r. spectrum was diffuse and showed no characteristic absorption bands.

Thiocholesterol.—Cholesteryl chloride was treated with a large excess of sodium thiocyanate in ethanol [20] except that crystallisation from ethanol–ethyl acetate was unsuccessful since the excess of sodium thiocyanate separated with the product. However, by extracting the crude solid with chloroform and filtering off the sodium thiocyanate, evaporation of the filtrate gave cholesteryl thiocyanate (75%), m.p. 126—128° (lit.,[20] 126—128°). Cholesteryl thiocyanate was reduced by lithium aluminium hydride [21] to give thiocholesterol (80%), m.p. 95—97° (lit.,[21] 98—99·5°).

Attempted Phosphorylation of Thiocholesterol (a) *With phosphorus oxychloride*. Treatment with the reagent in pyridine–acetone for 4 hr. at 0° gave unchanged thiocholesterol.

(b) *With pyrophosphoryl chloride*. A solution of thiocholesterol in ether or boiling ethyl acetate gave only starting material.

Attempted Hydrolysis of Cholesteryl Thionophosphorodichloridate.—(a) *With aqueous tetrahydrofuran*. Cholesteryl thionophosphorodichloridate (2 g.) was boiled with tetrahydrofuran (20 c.c.) and water (5 c.c.) for ½ hr.; hydrogen sulphide was evolved. The solvent was removed from the reaction mixture to leave a white solid (100 mg.), m.p. 170—175° (Found: C, 73·9; H, 10·1; Cl, 5·1; P, 4·6; S, 4·6. Cholesteryl thionophosphato, $C_{27}H_{47}O_3PS$ requires C, 67·3; H, 9·75; P, 6·4; S, 6·6%).

(b) *With boiling aqueous dioxan*. Treatment for 3 hr. gave a solid product (250 mg.), m.p. 74—79° (Found: C, 77·9; H, 10·8; Cl, 4·5; P, 0·6; S, 2·6%).

[20] G. L. O'Connor and H. R. Nace, *J. Amer. Chem. Soc.*, 1953, **75**, 2118.

[21] J. Strating and H. J. Backer, *Rec. Trav. chim.*, 1950, **69**, 638.

(c) *With boiling ethanolic potassium hydroxide.*[17] Treatment for 3 hr. gave a solid product (1·1 g.), m.p. 100° (from ethanol) (Found: C, 70·8; H, 10·6; P, 6·5; S, 5·2. *O*-cholesteryl *OO*-diethyl thiophosphate, $C_{31}H_{55}O_3PS$ requires C, 69·4; H, 10·2; P, 5·8; S, 5·95%).

Attempted hydrolyses with boiling water (3 hr.), and 5% aqueous sodium hydroxide at room temperature (14 days) gave unchanged cholesteryl thionophosphorodichloridate.

Cholesteryl Dihydrogen Phosphate.—*Method* (a); *with water.* Cholesteryl phosphorodichloridate (10 g.) was boiled under reflux with water (150 c.c.) for 4 hr. The suspension was treated with tetrahydrofuran (100 c.c.) and the mixture was evaporated under reduced pressure. The residue was recrystallised twice from dioxan to give cholesteryl dihydrogen phosphate as platelets (6·0 g., 65%), m.p. 186—188° (Found: C, 69·3; H, 10·0; P, 6·7. Calc. for $C_{27}H_{47}O_4P$: C, 69·5; H, 10·2; P, 6·6%), $\nu_{max.}$ 1270 (P=O), 1050 (P–O–C), 2350 (P–OH) [16h] cm.$^{-1}$. Treatment with cyclohexylamine in warm tetrahydrofuran gave the *monocyclohexylammonium salt* as needles, m.p. 215° (Found: C, 69·6; H, 10·6; N, 3·0; P, 4·4. $C_{33}H_{60}NO_4P$ requires C, 70·1; H, 10·6; N, 2·5; P, 4·7%).

Method (b); *with aqueous dioxan.* Cholesteryl phosphorodichloridate (10 g.) was boiled under reflux with dioxan (200 c.c.)–water (20 c.c.) for 4 hr. The solution was cooled, when cholesteryl dihydrogen phosphate crystallised as plates (4·3 g., 46%), m.p. 186°. Evaporation of the filtrate gave a residue, which crystallised from ethanol to give cholesteryl chloride (1·1 g., 12%), m.p. 95° (lit.,[18a] 97°).

Other methods for the hydrolysis of cholesteryl phosphorodichloridate were: boiling with 20% aqueous acetone for 3 hr. to give cholesteryl dihydrogen phosphate (35%) and cholesteryl chloride (6%); boiling with 20% aqueous pyridine (3 hr.) to give P^1,P^2-*dicholesteryl pyrophosphate* (85%), m.p. 143—145° (Found: C, 70·5; H, 10·2; P, 6·9. $C_{54}H_{92}O_7P_2$ requires C, 70·9; H, 10·1; P, 6·8%); boiling with 20% aqueous triethylamine (2 hr.) to give triethylamine hydrochloride and P^1,P^2-dicholesteryl pyrophosphate (60%), m.p. 140—142° (identical i.r. spectrum to that of P^1,P^2-dicholesteryl pyrophosphate obtained by the action of dicyclohexylcarbodi-imide on cholesteryl dihydrogen phosphate; or by selective hydrolysis of cholesteryl phosphorodichloridate [22]); boiling with 20% aqueous lithium hydroxide (¼ hr.) to give *dilithium cholesteryl phosphate* (50%), m.p. 190° (Found: C, 68·1; H, 9·4; P, 6·3. $C_{27}H_{45}Li_2O_4P$ requires C, 67·7; H, 9·4; P, 6·5%).

Cholesteryl Dimethyl Phosphate (with D. H. Wakeford).—Cholesteryl phosphorodichloridate (400 mg.) was warmed with methanol (30 c.c.) and pyridine (0·3 c.c.) for 10 min. and was then set aside for 16 hr. at room temperature. The solvent was removed under reduced pressure to give an oil which was treated with ether at 0°; pyridine hydrochloride (100 mg.) was filtered off and the filtrate was washed with water and then evaporated. The residue crystallised from pentane to give the *dimethyl phosphate* (320 mg.), m.p. 127—128° (Found: C, 70·0; H, 10·3; P, 6·0. $C_{29}H_{51}O_4P$ requires C, 70·4; H, 10·3; P, 6·3%), $\nu_{max.}$ 1280 (P=O), 1190 (P–OMe), 1040 (P–O–C) cm.$^{-1}$. The same product was obtained by treatment of cholesteryl phosphorodichloridate with sodium methoxide in methanol.

Cholestanyl Dihydrogen Phosphate.—Cholestanyl phosphorodichloridate (400 mg.) was boiled under reflux with 10% aqueous dioxan (10 c.c.) for 3 hr. The solution was cooled to give *cholestanyl dihydrogen phosphate* as plates (150 mg.), m.p. 172—173° (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.$^{-1}$.

Dicholesteryl Hydrogen Phosphate.—Dicholesteryl phosphorochloridate (16 g.) was boiled under reflux with water (250 c.c.) for 24 hr. The precipitate was collected and boiled with glacial acetic acid (100 c.c.) for 3 hr. to give dicholesteryl hydrogen phosphate as an amorphous solid (10 g.), m.p. 210° (lit.,[23] 208°; the material reported,[2] m.p. 186°, was almost certainly cholesteryl dihydrogen phosphate) (Found: C, 77·8; H, 11·2; P, 4·1. Calc. for $C_{54}H_{91}O_4P$: C, 77·7; H, 10·9; P, 3·7%), $\nu_{max.}$ 1230 (P=O), 1050 (P–O–C), 2300 (P–OH) cm.$^{-1}$. Treatment with cyclohexylamine in warm tetrahydrofuran gave the *cyclohexylammonium salt* as needles, m.p. 230° (Found: C, 77·1; H, 11·1; N, 1·6. $C_{60}H_{104}NO_4P$ requires C, 77·2; H, 11·2; N, 1·5%).

Ergosteryl Dihydrogen Phosphate.—Ergosteryl phosphorodichloridate (5 g.) was hydrolysed with boiling water as described for the preparation of cholesteryl dihydrogen phosphate. Two recrystallisations from anhydrous dioxan gave ergosteryl dihydrogen phosphate as pale yellow plates (2·3 g., 49%), m.p. 160° (lit.,[7] 165—168°) (Found: C, 70·1; H, 9·4; P, 6·2. Calc. for $C_{28}H_{45}O_4P$: C, 70·5; H, 9·4; P, 6·5%), $\nu_{max.}$ 1260 (P=O), 1600 (conj. C=C), 1060 (P–O–C), and 2350 (P–OH) cm.$^{-1}$.

Lanosteryl Dihydrogen Phosphate.—Lanosteryl phosphorodichloridate (500 mg.) was boiled with aqueous dioxan, as described in the preparation of cholesteryl phosphate [method (b)] to give *lanosteryl dihydrogen phosphate* as platelets (380 mg., 78%), m.p. 204—205° (Found: C, 70·4; H, 10·0; P, 6·5. $C_{30}H_{51}O_4P$ requires C, 70·7; H, 10·6; P, 6·1%).

Lanosteryl Dimethyl Phosphate (with D. H. Wakeford).—Lanosteryl phosphorodichloridate (400 mg.) was warmed with methanol for 15 min., and was then set aside for 1 week at room temperature. The solution was cooled to give the *dimethyl phosphate* as needles (320 mg.), m.p. 150° (Found: C, 71·8; H, 10·5; P, 5·5. $C_{32}H_{55}O_4P$ requires C, 72·0; H, 10·4; P, 5·8%).

Cholesteryl Diphenyl Phosphate.—Cholesterol (5 g.) was treated with diphenylphosphorochloridate in pyridine as described in ref. 5 to give the diphenyl phosphate as needles (from light petroleum) (5·6 g.), m.p. 117° (lit.,[5] 114—116°) $\nu_{max.}$ 1280 (P=O), 1190 [P–O–C (aryl)], 1020 [P–O–C (alk)], 3060 (arom. C–H) cm.$^{-1}$.

Cholestanyl Diphenyl Phosphate.—Cholestanol (5 g.), by similar treatment with diphenyl phosphorochloridate, gave the *diphenyl phosphate* as microprisms from acetone (5·2 g.), m.p. 89° (Found: C, 75·2; H, 9·0; P, 4·5. $C_{39}H_{57}O_4P$ requires C, 75·5; H, 9·2; P, 5·0%).

Ergosteryl Diphenyl Phosphate.—Ergosterol (5 g.) similarly gave the diphenyl phosphate as pale yellow needles (from light petroleum) (3·4 g.), m.p. 104° (lit.,[5] 106—107°) (Found: C, 76·3; H, 8·1; P, 4·4. Calc. for $C_{40}H_{53}O_4P$: C, 76·4; H, 8·4; P, 4·9%).

Ergosteryl Dimethyl Phosphate.—Ergosteryl phosphorodichloridate was boiled under reflux with sodium methoxide in methanol for 2 hr. to give the *dimethyl phosphate* as platelets, m.p. 153—154° (Found: C, 71·4; H, 9·5; P, 6·4. $C_{30}H_{49}O_4P$ requires C, 71·6; H, 9·7; P, 6·4%).

[22] R. J. Cremlyn and N. A. Olsson, unpublished observations.
[23] K. Zeile and W. Kruckenberg, *Ber.*, 1942, **75**, 1127.

Cholesteryl Thionodimethyl Phosphate.—Cholesteryl thionophosphorodichloridate (1 g.) was boiled under reflux with sodium (0·1 g.) in absolute ethanol (45 c.c.) for 2½ hr. The filtrate, after removal of sodium chloride, gave the *thionodiethyl phosphate* as pale yellow plates (0·8 g.), m.p. 100° (Found: C, 69·6; H, 10·0; P, 6·0; S, 6·45. $C_{31}H_{55}O_3PS$ requires C, 69·4; H, 10·2; P, 5·8; S, 5·95%).

Tricholesteryl Phosphate.—Dicholesteryl phosphorochloridate was heated with cholesterol in dioxan in the presence of pyridine for 20 hr. to give *tricholesteryl phosphate* as plates, m.p. 210—212° (Found: C, 80·6; H, 11·0; P, 2·8. $C_{81}H_{135}O_4P$ requires C, 80·9; H, 11·2; P, 2·6%).

6β-*Hydroxy*-3,5-*cycloergosta*-7,22-*diene.*—Ergosteryl diphenyl phosphate (2 g.) was boiled under reflux with acetone (500 c.c.), water (100 c.c.), and potassium hydrogen carbonate (1 g.) for 6 hr. The solution was concentrated until it was turbid; it was then cooled and the precipitate was filtered off and recrystallised from acetone to give the cycloergostadiene as needles (1 g.), m.p. 124—126° (lit.,[24] 129—130°).

Cholesteryl diphenyl phosphate after similar treatment for 20 hr. gave only unchanged starting material.

OO-*Dicholesteryl Hydrogen Phosphorodithioate.*—A solution of cholesterol (5 g.) in carbon disulphide (30 c.c.) was treated with phosphorus pentasulphide (0·73 g.) and the suspension was boiled under reflux for 3 hr. The solution was cooled at 0° overnight to give OO-*dicholesteryl hydrogen phosphorodithioate* as needles (4·0 g., 71%), m.p. 192—193° (Found: C, 74·4; H, 10·5; P, 3·6; S, 8·0. $C_{54}H_{91}O_2PS_2$ requires C, 74·8; H, 10·6; P, 3·6; S, 7·4%), $\nu_{max.}$ 980 (P–O–C), 740, 680 (P=S) cm.$^{-1}$.

OO-*Dicholesteryl Sodium Phosphorodithioate.*—*OO*-Dicholesteryl hydrogen phosphorodithioate (2 g.) was dissolved in warm xylene and sodium (0·1 g.) was added; a voluminous white precipitate was formed. This was filtered off and recrystallised from ethanol to give OO-*dicholesteryl sodium phosphorodithioate* as plates (1·5 g.), m.p. 267° (Found: C, 72·0; H, 9·6; P, 3·5; S, 7·5. $C_{54}H_{90}NaO_2PS_2$ requires C, 73·0; H, 10·1; P, 3·5; S, 7·2%).

OO-*Dicholesteryl* S-*Methyl Phosphorodithioate.*—*OO*-Dicholesteryl sodium phosphorodithioate (500 mg.) was dissolved in hot ethanol (25 c.c.) and dimethyl sulphate (2 c.c.). The precipitate was collected to give the S-*methyl derivative* (250 mg.), m.p. 188° (Found: P, 3·3; S, 7·6. $C_{55}H_{93}O_2PS_2$ requires P, 3·5; S, 7·3%); n.m.r. (CCl_4) showed τ 7·7, *J* 16 c./sec. (P–S–CH_3) and the steroid 'envelope' between τ 10 and 8·32. There was no peak at τ 6·23 indicating the absence of the P–OMe group. These assignments were confirmed by the n.m.r. spectrum (CCl_4) of *OO*-dimethyl *S*-methyl phosphorodithioate which showed doublets at τ 6·25, *J* 14 c./sec. (P–OMe) and τ 7·7, *J* 16 c./sec. (P–SMe).

O-*Tetracholesteryl Dithiophosphoryl Disulphide.*—*OO*-Dicholesteryl sodium phosphorodithioate (1·0 g.) was treated with an excess of a 10% solution of bromine in warm ethanol until a faint permanent yellow colour was obtained. The precipitate was recrystallised from benzene to give the *disulphide* as small prisms (0·75 g.), m.p. 203—204° (Found: C, 74·5; H, 10·1; P, 3·6; S, 8·0. $C_{108}H_{180}O_4PS_4$ requires C, 74·8; H, 10·5; P, 3·5; S, 7·4%). Titration of the disulphide in tetrahydrofuran with an automatic titrimeter with 0·1N-tetra-n-butylammonium hydroxide in toluene–methanol showed the absence of a free thiophosphate group by comparison with the titration of *OO*-dicholesteryl dithiophosphate.

Reaction of Cholesteryl Phosphorodichloridate with Methanol (with D. H. Wakeford).—Cholesteryl phosphorodichloridate (1·3 g.) was heated with methanol (50 c.c.) until all the solid had dissolved. The solution was cooled to give cholesteryl methyl ether (300 mg.), m.p. 80—82° (lit.,[18e] 83—84°), $\nu_{max.}$ 1120 (C–O–C). The n.m.r. spectrum in deuteriochloroform showed signals at τ 4·6 (vinylic H) and 6·65 (OMe). Evaporation of the filtrate gave a waxy solid (1·0 g.). The n.m.r. spectrum ($CDCl_3$) showed a strong doublet at τ 6·23, *J* 16 c./sec. (P–OMe) (*cf.* ref. 9). Cholesteryl dimethyl phosphate (from diazomethane–cholesteryl dihydrogen phosphate) had an identical n.m.r. spectrum.

Reaction of Egosteryl Phosphorodichloridate with Methanol.—Ergosteryl phosphorodichloridate (100 mg.) was heated with methanol (25 c.c.) until dissolution was complete The solution was cooled to give 3,5-cycloergosta-6,8(14),22-triene (50 mg.) as needles, m.p. 94—97°, $\lambda_{max.}$ (cyclohexane) 261 mμ, (ε 23,400), $\nu_{max.}$ 3040 cm.$^{-1}$ (cyclopropane ring),[16f] $[\alpha]_D^{20}$ ($CHCl_3$) +85° (lit.,[18c] 102°, 261 mμ, 26,800, $[\alpha]_D$ +92°). The same compound was obtained in an analogous experiment in the presence of pyridine.

Decomposition of Cholesteryl Phosphorodichloridate in Various Solvents.—(a) *In dioxan.* Cholesteryl phosphorodichloridate (2 g.) was boiled under reflux with anhydrous dioxan (100 c.c.) for 1 hr. The brown solution was evaporated under reduced pressure and the black oil was dissolved in chloroform and chromatographed on aluminium oxide (150 g.). Elution with light petroleum (b.p. 60—80°) gave white plates (877 mg.), m.p. 76°, $\lambda_{max.}$ ($CHCl_3$) 235 mμ (ε 19,000) $[\alpha]_D^{20}$ −105° ($CHCl_3$) (probably mainly cholesta-3,5-diene (lit.,[18b] 80°, $\lambda_{max.}$ 235 mμ (ε 20,000), $[\alpha]_D$ −123°).

(b) *In ethyl acetate.* Cholesteryl phosphorodichloridate (2 g.) was boiled under reflux with ethyl acetate (100 c.c.) for 4 hr. to give a red oil (2 g.); this was treated as described in (a). Elution with light petroleum (b.p. 60—80°) gave a white solid (200 mg.), m.p. 75°, $\lambda_{max.}$ (cyclohexane) 235 mμ (ε 15,700) (probably mainly cholesta-3,5-diene. Elution with benzene–light petroleum (1 : 1) gave a solid (687 mg.), m.p. 84—86° (lit.,[18f] 116°, mixed m.p. with cholesteryl acetate 113—116°). The i.r. spectrum was identical to that of cholesteryl acetate, $\nu_{max.}$ 1740 cm.$^{-1}$ (CO); n.m.r. spectrum ($CDCl_3$) showed τ 7·83 (COMe).

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