882. *Some Cholesteryl Phosphates.*

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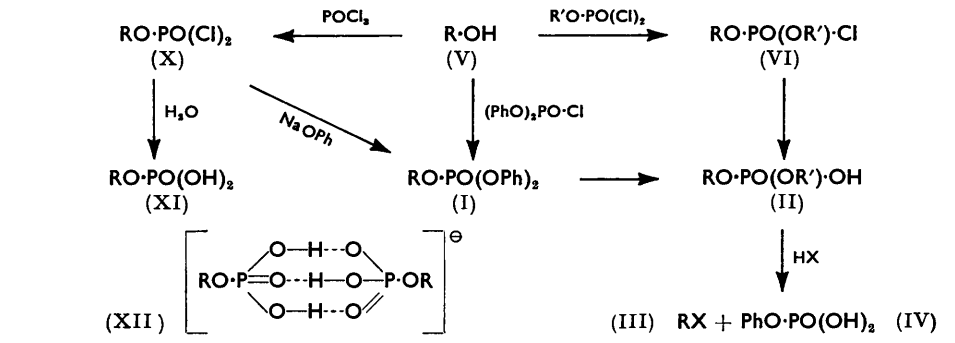
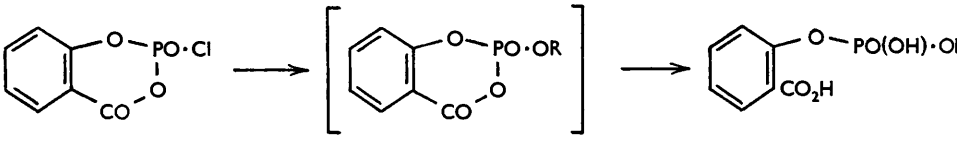
Cholesteryl diphenyl phosphate with aqueous-alcoholic alkali undergoes both hydrolysis and ethanolysis. The major products are cholesteryl phenyl and cholesteryl ethyl hydrogen phosphates. The structure of these has been established by independent syntheses. The former had been isolated previously when it was believed to be cholesteryl dihydrogen phosphate. The dihydrogen phosphate itself, conveniently prepared from hydrolysis of cholesteryl phosphorodichloridate, forms a stable " half " pyridine salt.

The action of aqueous ethanolic potassium hydroxide on cholesteryl diphenyl phosphate (I) was earlier [1] found to give about 1·75 mol. of phenol, and an acid (m. p. 162°) was isolated. These results have been confirmed but it is now clear that the acid is cholesteryl phenyl hydrogen phosphate (II; R′ = Ph) and not the dihydrogen phosphate as suggested earlier. Two mol. of phenol are not produced even on prolonged reaction, and no phenol is formed from cholesteryl phenyl hydrogen phosphate under these conditions, the anion of the latter being stable to alkali.[cf. 2] The implication that base-catalysed ethanolysis [cf. 3, 4] of cholesteryl diphenyl phosphate was accounting for the liberation of a considerable part of the phenol has now been confirmed by the isolation of cholesteryl ethyl hydrogen phosphate (II; R′ = Et) from the products. Also, phenol was rapidly formed from cholesteryl diphenyl phosphate in cold *anhydrous* potassium ethoxide solution.

Solvolysis of cholesteryl phenyl hydrogen phosphate with hydrogen chloride in acetic acid gives 3β-chlorocholest-5-ene (III; X = Cl) and phenyl dihydrogen phosphate (IV), whilst refluxing it with acetic acid yields 3β-acetoxycholest-5-ene (III; X = OAc) and phenyl dihydrogen phosphate, with retention of configuration at $C_{(3)}$ in each case.[cf. 1]





(VII) (VIII) (IX)

R = Cholesteryl

Cholesteryl phenyl hydrogen phosphate was synthesised as follows. Phenyl phosphorodichloridate with cholesterol (V) in 2 : 6-lutidine gave cholesteryl phenyl phosphorochloridate (VI; R′ = Ph). The latter was hydrolysed when refluxed with aqueous

[1] Turnbull and Wilson, *J.*, 1954, 2301.
[2] Barnard, Bunton, Llewellyn, Oldham, Silver, and Vernon, *Chem. and Ind.*, 1955, 760.
[3] Morel, *Compt. rend.*, 1899, **128**, 507.
[4] Rueggerberg and Chernack, *J. Amer. Chem. Soc.*, 1948, **70**, 1802.

*iso*propyl alcohol affording cholesteryl phenyl hydrogen phosphate (II; R′ = Ph). Alternatively the phosphorochloridate (VI; R′ = Ph) was treated with tetrahydropyran-2-ol: the resulting syrup, presumably cholesteryl phenyl tetrahydro-2-pyranyl phosphate, lost dihydropyran when heated, yielding cholesteryl phenyl hydrogen phosphate. This method may be useful for the hydrolysis of other phosphorochloridates of high molecular weight which often yield gelatinous products by direct hydrolysis. Cholesteryl ethyl hydrogen phosphate (II; R′ = Et) was synthesised from ethyl phosphorodichloridate and cholesterol, the intermediate phosphorochloridate (VI; R′ = Et) being indirectly hydrolysed with *tert.*-butyl alcohol.

Most of the known phosphorylation methods [1] were considered unsuitable for the preparation of cholesteryl dihydrogen phosphate. In view of discrepancies in the literature on the properties of this compound an attempt was made to synthesise it by using anhydro-(*o*-carboxyphenyl phosphorochloridate) (VII) as phosphorylating agent. The latter, prepared by refluxing salicylic acid with phosphorus oxychloride, reacted rapidly with cholesterol in 2:6-lutidine yielding cholesteryl *o*-carboxyphenyl hydrogen phosphate (IX), the corresponding anhydride (VIII) probably being an intermediate. Attempts to hydrolyse the phosphate (IX) to cholesteryl dihydrogen phosphate (cf. Chanley and Gindler [5]) failed. It resisted hydrolysis in alkaline or weakly acid aqueous conditions; solvolysis occurred with hydrogen chloride in acetic acid affording 3β-chlorocholest-5-ene.

Hydrolysis of cholesteryl phosphorodichloridate (X) has been claimed to yield cholesteryl dihydrogen phosphate.[6] This has now been confirmed, although the yield of the dihydrogen phosphate is poor, and considerable attention to detail is essential. Cholesteryl phosphorodichloridate, prepared from cholesterol and phosphorus oxychloride in pyridine,[6] was characterised by reaction with sodium phenoxide to give cholesteryl diphenyl phosphate (I). Hydrolysis of the pyridine-free phosphorodichloridate yielded crystalline cholesteryl dihydrogen phosphate (XI). Failure to remove pyridine, as in the procedure of Wagner-Jauregg, Lennartz, and Kothny,[7] invariably yielded the salt $C_5H_5N,2RO{\cdot}PO(OH)_2,H_2O$ which is stable in the presence of dilute mineral acids, but is decomposed by aqueous potassium hydroxide with the liberation of pyridine. This salt was independently made from cholesteryl dihydrogen phosphate and pyridine. The formation of such "half" salts seems to be characteristic of alkyl dihydrogen phosphates.[1, 9, 10] The latter are dimeric in solution,[1, 8] and may tend to yield three-dimensional hydrogen-bonded anions (XII; cf. the similar two-dimensional structures already suggested for these compounds [11]).

Cholesteryl dihydrogen phosphate, as prepared by this method, was obtained as well-defined crystals (m. p. 181°), but other higher-melting and less crystalline forms were sometimes obtained. In view of this and of the ready formation of the pyridine salt, it is not surprising that there is considerable variation in melting points previously recorded for this compound. Cholesteryl dihydrogen phosphate titrated as a dibasic acid in aqueous ethanol. In water, gels were readily formed making the interpretation of potentiometric titration results difficult. With hydrogen chloride in acetic acid cholesteryl dihydrogen phosphate yielded 3β-chlorocholest-5-ene.

Experimental

$[\alpha]_D$ were measured in chloroform unless otherwise specified. Ultraviolet absorption maxima were determined in ethanol.

2 : 6-Lutidine was purified as described by Biddiscombe *et al.*[14]

Alkaline Hydrolysis of Cholesteryl Diphenyl Phosphate.—Cholesteryl diphenyl phosphate (2·4 g.), ethanol (120 c.c.), and 4N-potassium hydroxide (30 c.c.) were refluxed gently for 19 hr. The product, worked up as in Turnbull and Wilson's procedure,[1] yielded *cholesteryl phenyl hydrogen phosphate* (1·0 g.), which formed hygroscopic platelets (from ethyl acetate), m. p. 160°,

[5] Chanley and Gindler, *J. Amer. Chem. Soc.*, 1953, **75**, 4035.
[6] Von Euler, Wolf, and Hellström, *Ber.*, 1929, **62**, 2451.
[7] Wagner-Jauregg, Lennartz, and Kothny, *Ber.*, 1941, **74**, 1513.
[8] Wagner-Jauregg, *J. Org. Chem.*, 1954, **19**, 1483.
[9] Hunter, Roberts, and Kester, *J. Amer. Chem. Soc.*, 1948, **70**, 3244.
[10] Brown, Malkin, and Maliphant, *J.*, 1955, 1584.
[11] Wagner-Jauregg and Wildermuth, *Ber.*, 1944, **77**, 481.

$[\alpha]_D$ −28°, $\lambda_{max.}$ 264 (ε 524) (Found: C, 71·0; H, 10·3; P, 6·0. $C_{33}H_{51}O_4P,H_2O$ requires C, 70·7; H, 9·5; P, 5·5%).

Concentration of the mother-liquors afforded a white solid (700 mg.) and a syrup (400 mg.). Recrystallisation of the solid from benzene–light petroleum then from ethyl acetate yielded cholesteryl ethyl hydrogen phosphate (350 mg.), m. p. 156—157° undepressed by admixture with the synthetic material (below).

In another experiment cholesteryl diphenyl phosphate (49 mg.) was similarly treated with alkali. Liberated phenol (*ca.* 1·6 mol.) was estimated spectrophotometrically. Cholesteryl phenyl hydrogen phosphate was recovered after similar treatment with alkali during 3 days.

Reaction of Cholesteryl Phenyl Hydrogen Phosphate with Hydrogen Chloride.—The phenyl hydrogen phosphate (200 mg.), acetic acid (5 c.c.), and concentrated hydrochloric acid (0·5 c.c.) were warmed (100°) for 10 min. The product was diluted with water and gave 3β-chlorocholest-5-ene (150 mg.; m. p. and mixed m. p. 88—90°). The filtrates when treated with aqueous *cyclo*hexylamine afforded needles of di(*cyclo*hexylammonium) phenyl phosphate, m. p. 212° (decomp.; undepressed by admixture with an authentic specimen, m. p. 212° decomp.[15]).

Reaction of Cholesteryl Phenyl Hydrogen Phosphate with Acetic Acid.—The phenyl hydrogen phosphate (425 mg.) and acetic acid (6 c.c.) were refluxed for 27 hr. The product, treated as in the foregoing experiment, yielded 3β-acetoxycholest-5-ene (310 mg.; m. p. 112°) and di(*cyclo*hexylammonium) phenyl phosphate.

Reaction of Cholesteryl Diphenyl Phosphate with Acetic Acid.—The diphenyl phosphate (100 mg.) and acetic acid (3 c.c.) were refluxed for 24 hr. The product, treated as in the foregoing experiments, yielded 3β-acetoxycholest-5-ene (50 mg.; m. p. 113°) and *cyclo*hexylammonium diphenyl phosphate, m. p. and mixed m. p. 197—199° (Turnbull and Wilson [1]).

Synthesis of Cholesteryl Phenyl Hydrogen Phosphate.—Phenyl phosphorodichloridate (4·2 g.), 2 : 6-lutidine (2·7 g.), and benzene (10 c.c.) were mixed, and cholesterol (7·7 g.) in benzene (25 c.c.) gradually added. The mixture was warmed to 50°, stirred for 4 hr. at room temperature, and filtered from 2 : 6-lutidine hydrochloride (2·9 g.). The filtrate was divided into two equal portions (A and B). Portion (A) was washed with dilute hydrochloric acid, and refluxed for 30 min. with *iso*propyl alcohol (35 c.c.) and water (3 c.c.), and evaporated to dryness in a vacuum; the residue was crystallised first from benzene–light petroleum and then from ethyl acetate affording cholesteryl phenyl hydrogen phosphate (2·6 g.; m. p. 160—162° undepressed on admixture with a specimen prepared by the foregoing hydrolysis of the diphenyl phosphate). (B) was mixed with tetrahydropyran-2-ol (1·1 g.) and 2 : 6-lutidine (1·1 g.), and set aside for 40 hr. The product was evaporated leaving a syrup, presumably cholesteryl phenyl tetrahydropyran-2-yl phosphate, which was miscible with light petroleum, and decomposed at 100° during 2 hr. affording cholesteryl phenyl hydrogen phosphate (3·0 g.; m. p. and mixed m. p. 160°).

Synthesis of Cholesteryl Ethyl Hydrogen Phosphate.—Cholesterol (38·7 g.) in benzene (150 c.c.) was added to ethyl phosphorodichloridate (16·3 g.) and 2 : 6-lutidine (10·7 g.) in benzene (100 c.c.). The cloudy solution was warmed to 40°, set aside for 18 hr., and lutidine hydrochloride (12 g.) removed. *tert.*-Butyl alcohol (100 c.c.) was added, the solution refluxed for 30 min., water (4·0 c.c.) added, and the mixture evaporated in a vacuum. The residue was dissolved in excess of aqueous ethanolic potassium hydroxide, precipitated as a flocculent solid by acidification, dried, and recrystallised from benzene–light petroleum and then from ethyl acetate affording a *substance* as prisms (8·0 g.), m. p. 123—124° (Found: C, 76·1; H, 11·0; P, 3·2. Calc. for $C_{54}H_{91}O_4P,H_2O$: C, 76·1; H, 11·0; P, 3·6%). Titration of an aqueous ethanolic solution with aqueous potassium hydroxide (phenolphthalein) gave an equivalent weight of 852 (calc. for one acidic hydrogen, 853).

The mother-liquors from the foregoing recrystallisation were evaporated and treated with ethyl acetate, the solid (6·0 g.) was recrystallised from benzene–light petroleum then from ethyl acetate to give *cholesteryl ethyl hydrogen phosphate*, m. p. 155—158° (Found: C, 70·7; H, 10·2%; equiv., 480. $C_{29}H_{51}O_4P$ requires C, 70·4; H, 10·4%; equiv., 495).

Anhydro-(o-*carboxyphenyl Phosphorochloridate*).—Salicylic acid (69·0 g.) and phosphorus oxychloride (76·7 g.) were gradually heated to 150°, and maintained at 150° for 2 hr. The viscous product was distilled and the fraction, b. p. 116—125°/0·02 mm., crystallised from carbon tetrachloride affording prisms (39·6 g.), m. p. 90—93° (Found: C, 38·4; H, 2·2. Calc. for $C_7H_4O_4PCl$: C, 38·5; H, 1·85%). The substance is probably the same as that (m. p. 80°) described by Couper [12] and Anschütz.[13]

[12] Couper, *Compt. rend.*, 1858, **46**, 1107.
[13] Anschütz, *Annalen*, 1885, **228**, 308.

Cholesteryl o-Carboxyphenyl Hydrogen Phosphate.—The foregoing phosphorochloridate (8·0 g.) was dissolved in chloroform (30 c.c.), then 2 : 6-lutidine (4·0 g.) and cholesterol (14·2 g.) in chloroform (30 c.c.) were added. The mixture was set aside overnight, washed with water, dried (Na_2SO_4), and evaporated. The syrupy residue was diluted with benzene, excess of light petroleum added, and the precipitate crystallised from benzene–light petroleum affording the *phosphate* (2·6 g.), m. p. 141—142°, $[\alpha]_D$ −20° (ethanol), which was easily soluble in dilute aqueous sodium hydroxide (Found: C, 67·7; H, 8·5%; equiv. (by potentiometric titration), 296. $C_{34}H_{51}O_6P,H_2O$ requires C, 67·5; H, 8·8%; equiv., 302).

Reaction of Cholesteryl o-Carboxyphenyl Hydrogen Phosphate with Hydrogen Chloride.—The foregoing hydrogen phosphate (165 mg.) was dissolved in acetic acid (3 c.c.) and concentrated hydrochloric acid (0·3 c.c.) added. The solution was heated at 100° for 10 min. and diluted with water affording 3-β-chlorocholest-5-ene (m. p. 92—93°). The filtrates gave a strong purple coloration with aqueous ferric chloride.

Cholesteryl Phosphorodichloridate.—The crude pyridine-containing substance prepared from cholesterol (20 g.) by von Euler, Wolf, and Hellström's method [6] was extracted with hot light petroleum (b. p. 60—80°). The extracts deposited a solid which was recrystallised from light petroleum affording the phosphorodichloridate (7·5 g.), m. p. 110° (decomp.), $[\alpha]_D$ −31° [Von Euler, Wolf, and Hellström give m. p. 122° (decomp.)]

Cholesteryl Diphenyl Phosphate.—The foregoing phosphorodichloridate (530 mg.) was triturated with a solution of phenol (1 g.) and sodium ethoxide [from sodium (54 mg.) and ethanol (2 c.c.)]. Excess of dilute aqueous potassium hydroxide was added; the precipitate was recrystallised from aqueous acetone affording the diphenyl phosphate (520 mg.), m. p. 113° undepressed on admixture with a specimen prepared by Turnbull and Wilson's method.[1]

Cholesteryl Dihydrogen Phosphate.—Cholesterol (20 g.) was converted into the crude phosphorodichloridate by the foregoing method, and the product hydrolysed, without further purification, by refluxing it for 1¼ hr. with water (600 c.c.). The flocculent precipitate (A) was dissolved in aqueous potassium hydroxide (0·35N; 300 c.c.), and the solution filtered through Amberlite resin IR-120(H) and evaporated to complete dryness in a vacuum over phosphoric oxide. The residue was refluxed with benzene (200 c.c.) and water (2 c.c.) for 4 hr., the solution filtered and evaporated until crystallisation began. The product (10·7 g.) was recrystallised from a mixture of acetone and moist carbon tetrachloride affording irregular plates, m. p. 181° (decomp.), $[\alpha]_D$ −21° (ethanol) [Found: C, 69·5; H, 10·3%; equiv. (by titration), 229, 236. Calc. for $C_{27}H_{47}O_4P$: C, 69·5; H, 10·15%; equiv., 233).

The dihydrogen phosphate is insoluble in warm, dry benzene, carbon tetrachloride, or chloroform, but dissolves in the presence of water. Removal of the water by azeotropic distillation causes immediate precipitation of the substance. The dihydrogen phosphate, as thus prepared, is soluble in aqueous potassium hydroxide, but is insoluble in aqueous sodium hydroxide. A less soluble, metastable form, m. p. 187°, [cf.16] was obtained by rapid drying of its aqueous gel.

" Half " Pyridine Salt of Cholesteryl Dihydrogen Phosphate.—A portion of the solid (A) from the foregoing experiment was recrystallised from benzene affording the " half " pyridine salt, m. p. 178° (with sintering and darkening), $[\alpha]_D$ −36° (Found: C, 68·4; H, 9·8; N, 1·3. $2RO{\cdot}PO(OH)_2,C_5H_5N,H_2O$ requires C, 68·8; H, 9·9; N, 1·4%). An identical compound was obtained from pure cholesteryl dihydrogen phosphate and aqueous pyridine. The substance was recovered when its solution in aqueous potassium hydroxide was acidified with hydrochloric acid.

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[14] Biddiscombe, Coulson, Handley, and Herington, *J.*, 1954, 1957.
[15] Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815.
[16] Neuberg and Jacobsohn, *Biochem. Z.*, 1928, **199**, 507.