Reactions of Diphenyl Phosphates of Δ^5*-Sterols.*

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Crystalline diphenyl phosphates are made from several sterols and diphenyl phosphorochloridate in pyridine. The solvolytic reactions of Δ^5-steryl diphenyl phosphates are interesting; thus cholesteryl diphenyl phosphate is hydrolysed by alkali to phenol and cholesteryl dihydrogen phosphate. An alternative mode of fission occurs with hydrogen chloride (or bromide) in warm acetic acid, and diphenyl hydrogen phosphate and 3β-chloro(bromo)cholest-5-ene are formed. 3β-Halogeno-Δ^5-steroids are obtained in high yields by these reactions, in which the configuration at $C_{(3)}$ is retained. Cholesteryl dihydrogen phosphate is associated, usually dimeric, and has been mistaken for *sym.*-dicholesteryl dihydrogen pyrophosphate.

DIRECT phosphorylation of sterols is not very satisfactory for preparative purposes (von Euler and Bernton, *Ber.*, 1927, **60**, 1720; von Euler, Wolf, and Hellström, *Ber.*, 1929, **62**, 2451; Plimmer and Burch, *J.*, 1929, 279, 292; Wagner-Jauregg, Lennartz, and Kothny, *Ber.*, 1941, **74**, 1513; Reichstein and Schindler, *Helv. Chim. Acta*, 1940, **23**, 669; Müller, Langerbeck, and Riedel, *Z. physiol. Chem.*, 1944, **281**, 29). Indirect phosphorylation using phosphoramidic chlorides has not been fully investigated; it has been used for the preparation of dicholesteryl hydrogen phosphate (Zeile and Kruckenberg, *Ber.*, 1942, **75**, 1127; cf. Zetsche and Buttiker, *Ber.*, 1940, **73**, 47). Phosphates have been made recently from steroid hormones, by using tetrabenzyl pyrophosphate (Roche Products, Atherton, and Todd, B.P., 674,087; *Chem. Abs.*, 1953, **47**, 6436).

We have obtained crystalline diphenyl phosphates from several sterols and diphenyl phosphorochloridate in pyridine (see Table). The solvolytic reactions of these products have been studied, one objective being a route to steryl phosphates.

Alkaline hydrolysis of cholesteryl diphenyl phosphate (I; R = cholesteryl) afforded phenol (2 equivs.) and cholesteryl dihydrogen phosphate (II), m. p. 162—163°. The absence of free cholesterol in the hydrolysate confirms the alkali-stability of alkyl hydrogen phosphates (*e.g.*, Kosolapoff, " Organophosphorus Compounds," Wiley, New York, 1950, p. 233; Davis and Ross, *J.*, 1952, 4299). The recorded m. p. of cholesteryl dihydrogen phosphate varies considerably; von Euler *et al.* (*locc. cit.*) give m. p. 195—196° and 193°, and Wagner-Jauregg *et al.* (*loc. cit.*), using von Euler's preparative method, obtained a product of m. p. 175°. The molecular weight of cholesteryl dihydrogen phosphate as usually determined is about twice the theoretical value. This association, also observed with dicholesteryl hydrogen phosphate (Zeile and Kruckenberg, *loc. cit.*), has led to the erroneous pyrophosphate formulation (III; R = cholesteryl) (Wagner-Jauregg *et al.*, *loc. cit.*), which appeared to be confirmed by the isolation of " half " sodium or potassium

salts (Wagner-Jauregg and Lennartz, *Ber.*, 1942, **75**, 178). However, alkyl dihydrogen phosphates commonly form " half " salts (*i.e.*, salts containing one atom of sodium for two molecules of acid) (Friedman and Seligman, *J. Amer. Chem. Soc.*, 1951, **73**, 5292; Wagner-Jauregg and Wildermuth, *Ber.*, 1944, **77**, 481). The electrometric titration of cholesteryl dihydrogen phosphate was difficult because of the solubilities of the substance and its salts. In methanol–benzene, only one acidic hydrogen was revealed, and this result does not therefore exclude the pyrophosphate structure (III). It is unlikely, however, that a pyrophosphate could have been formed or would have survived the strongly alkaline hydrolysis; also, our product has no well-defined infra-red absorption in the 930—950 cm.$^{-1}$ region, where the pyrophosphate P–O–P group often absorbs (cf. Bergmann, Littauer, and Pinchas, *J.*, 1952, 847).

An alternative mode of fission of cholesteryl diphenyl phosphate occurs with hydrogen chloride in warm acetic acid, and 3β-chlorocholestene (IV; R = cholesteryl, X = Cl) and diphenyl hydrogen phosphate (V) were isolated. The diphenyl phosphates of stigmasterol and dehydro*epi*androsterone, which are also Δ^5-compounds, behaved in the same way; similarly, hydrogen bromide yielded the corresponding 3β-bromo-compounds. The 3-halides were obtained in high yields, with retention of configuration at $C_{(3)}$. The solvent appears to play a critical rôle in these reactions, which did not occur when the acetic acid was replaced by dioxan. The reaction is probably facilitated by the tendency of the 3-substituent to separate as a stable diphenyl phosphate anion. Initial activation by formation of an oxonium salt (*e.g.*, VI) with protons may be necessary before dealkylation

$$\underset{\text{(I)}}{\text{RO·PO(OPh)}_2} \xrightarrow{\text{OH}^-} \underset{\text{(II)}}{\text{RO·PO(OH)}_2} + 2\text{PhOH}$$

$$\downarrow \text{HX}$$

$$\underset{\text{(III)}}{\text{RO·PO(OH)·O·PO(OH)·OR}} \qquad \underset{\text{(IV)}}{\text{RX}} + \underset{\text{(V)}}{\text{HO·PO(OPh)}_2} \qquad \underset{\text{H}\quad\text{(VI)}}{\text{R}\overset{+}{\text{O}}\text{·PO(OPh)}_2}$$

can occur (cf. Shoppee and Summers, *J.*, 1952, 3361); in dioxan, the solvent may be sufficiently basic to inhibit oxonium salt formation from steryl diphenyl phosphates. The tendency of alkyl phosphates to undergo ionic alkyl–oxygen fission is indicated, for example, by experiments on their dealkylation by hydrogen halides (Gerrard, Green, and Nutkins, *J.*, 1952, 4076; Blumenthal and Herbert, *Trans. Faraday Soc.*, 1945, **41**, 611), and by the reaction between benzyl phosphates and lithium chloride or tertiary amines (Clark and Todd, *J.*, 1950, 2030). Also, ionic elimination reactions occur in phosphates of β-hydroxycarbonyl compound (Linstead, Owen, and Webb, *J.*, 1953, 1211; Brown, Fried, and Todd, *Chem. and Ind.*, 1953, 352; Riley, Turnbull, and Wilson, *ibid.*, p. 1181).

The reaction of optically active alkyl phosphates with hydrogen halides normally gives alkyl halides with *inversion* of configuration (Gerrard, Green, and Nutkins, *loc. cit.*; Bevan, Brown, Gregory, and Malkin, *J.*, 1953, 127). The retention of configuration observed with the Δ^5-sterol derivatives is apparently anomalous. However, similar occurrences have been satisfactorily accounted for by Shoppee and his co-workers (*J.*, 1952, 3361 and earlier papers; cf. Landauer and Rydon, *J.*, 1953, 2224). There is convincing evidence that the 5 : 6-double bond in steroids can participate in reactions at $C_{(3)}$; as a result, either substitution with retention of configuration at $C_{(3)}$, or rearrangement to 6β-substituted *cyclo*steroids, occurs. In our experiments, *cyclo*steroids were not detected; they would have been rapidly converted under the conditions used into 3β-halogeno-Δ^5-steroids (cf. Shoppee and Summers, *loc. cit.*).

Experimental

$[\alpha]_D$ were measured in chloroform.

Cholesteryl Diphenyl Phosphate.—Dry cholesterol (2·5 g.) was dissolved in dry pyridine (25 c.c.) and diphenyl phosphorochloridate (2·0 g.) added at 0°. After 16 hr. at 45°, excess of ice was added. The crystals which soon separated were recrystallised from ethanol; for yield, etc., see Table. The procedures for making all the *diphenyl phosphates* were similar.

Alkaline Hydrolysis of Cholesteryl Diphenyl Phosphate.—The diphenyl phosphate (600 mg.), ethanol (30 c.c.), and 4N-potassium hydroxide (8 c.c.) were heated at 95° for 19 hr. The solution

was cooled, diluted with 60% ethanol, and filtered through Amberlite resin IR-120. The percolate was strongly acid and formed a gel when kept. Solvent was removed at 40° in a vacuum, and the solid residue (360 mg.) was crystallised from benzene–light petroleum (b. p. 40—60°), then from ethyl acetate, affording cholesteryl dihydrogen phosphate, white needles, m. p. 162—163°, $[\alpha]_D$ −30°, $\lambda_{max.}$ 263 (ε 1150) [Found: C, 71·0; H, 10·3%; *M* (ebullioscopic; benzene), 874; *M* (Rast), 720. Calc. for $C_{27}H_{47}O_4P$: C, 70·85; H, 10·15%; *M*, 467]. Electrometric titration (glass electrode) of a benzene solution with methanolic potassium hydroxide gave an equivalent weight of 507 (calc. for one acidic hydrogen, 467). In a further experiment, cholesteryl diphenyl phosphate (100 mg.) was similarly treated with alkali, and the ethanol removed. The residue consumed 40·0 c.c. of 0·042N-bromine water, corresponding to the presence of 1·75 mols. of phenol (also, the 2 : 4 : 6-tribromophenol formed was isolated). The phosphate was precipitated, less pure, by acidification of alkaline hydrolysates.

Reaction of Cholesteryl Diphenyl Phosphate with Hydrogen Chloride.—The diphenyl phosphate (200 mg.), acetic acid (2 c.c.), and concentrated hydrochloric acid (0·2 c.c.) were warmed (60°) for 20 min. The clear solution soon deposited an oil which crystallised on cooling. The product (150 mg.; m. p. 67—70°) recrystallised from glacial acetic acid, and gave 3β-chlorocholest-5-ene (85 mg.) as glistening plates, m. p. 92—93°, $[\alpha]_D^{20}$ −26·4° (Found: C, 79·8; H, 11·25. Calc. for $C_{27}H_{45}Cl$: C, 80·05; H, 11·1%). Beynon, Heilbron, and Spring (*J.*, 1936, 909) give m. p. 95°, $[\alpha]_D$ −27·4°. The aqueous liquors, after separation of the crude product, were evaporated to dryness and treated with aqueous *cyclo*hexylamine, affording colourless needles of *cyclo*hexylammonium diphenyl phosphate (25 mg.), m. p. 197—199° (Found: N, 4·25. Calc. for $C_{12}H_{11}O_4P,C_6H_{13}N$: N, 4·0%) (Corby, Kenner, and Todd, *J.*, 1952, 1241, give m. p. 198—199°).

Sterol	Diphenyl phosphate	
Cholesterol	Plates from methanol	M. p. 114—116°, $[\alpha]_D$ −9·2°, $\lambda_{max.}$ 261·5 (ε 660) (Found: C, 75·5; H, 9·0; P, 5·0. $C_{39}H_{55}O_4P$ requires C, 75·7; H, 9·0; P, 5·0%).
Stigmasterol	Needles from methanol	M. p. 99—101°, $[\alpha]_D$ −22°, $\lambda_{max.}$ 261·5 (ε 706) (Found: C, 76·5; H, 8·6. $C_{41}H_{57}O_4P$ requires C, 76·4; H, 8·9%).
Fucosterol	Needles from methanol	M. p. 80—81°, $[\alpha]_D$ −24°, $\lambda_{max.}$ 261·5 (ε 671) (Found: C, 76·7; H, 8·8. $C_{41}H_{57}O_4P$ requires C, 76·4; H, 8·9%).
Dehydro*epi*androsterone	Chromatographed,[1] needles from light petroleum (b. p. 60—80°)	M. p. 94—96° (Found: C, 71·1; H, 7·4. $C_{31}H_{37}O_5P$ requires C, 71·5; H, 7·2%).
5-Dihydroergosterol	Cryst. from methanol	M. p. 74—76°, $[\alpha]_D$ −12° (Found: C, 76·0; H, 8·7. $C_{40}H_{55}O_4P$ requires C, 76·2; H, 8·8%).
Zymosterol	Chromatographed,[2] colourless gum	Found: C, 75·6; H, 8·6. $C_{39}H_{53}O_4P$ requires C, 75·9; H, 8·7%.
Ergosterol	Cryst. from methanol	M. p. 106—107°, $[\alpha]_D$ −49° (Found: C, 76·7; H, 8·6. $C_{40}H_{53}O_4P$ requires C, 76·4; H, 8·5%).
7-Dehydrocholesterol	Small rosettes, chromatographed,[3] cryst. from methanol	M. p. 75—77°, $[\alpha]_D$ −31° (Found: C, 75·6; H, 8·6; $C_{39}H_{53}O_4P$ requires C, 75·9; H, 8·7%).
Dehydroergosterol	Chromatographed,[4] needles from methanol	M. p. 82—84° (Found: C, 76·3; H, 8·15. $C_{40}H_{51}O_4P$ requires C, 76·6; H, 8·2%).
Calciferol	Chromatographed, colourless gum (unstable)	Found: C, 75·0; H, 8·9. $C_{40}H_{53}O_4P$ requires C, 76·4; H, 8·5%. $\lambda_{max.}$ 264 (ε 15,150). Calciferol has $\lambda_{max.}$ 265 (ε 19,150).

[1] Eluted with 1 : 1 benzene–light petroleum. [2] Eluted with 2 : 3 benzene–light petroleum. [3] Eluted with 1 : 1 benzene–light petroleum. [4] Eluted with 1 : 1 benzene–light petroleum. Unstable.

3β-*Bromocholest-5-ene.*—Cholesteryl diphenyl phosphate (100 mg.) was warmed (60°) with glacial acetic acid and 48% aqueous hydrobromic acid. The product (70 mg.; m. p. 85—93°) formed glistening plates, m. p. 97—98°, $[\alpha]_D$ −18°, from acetic acid (Found: C, 72·6; H, 10·0. Calc. for $C_{27}H_{45}Br$: C, 72·2; H, 10·0%) (Beynon, Heilbron, and Spring, *loc. cit.*, give m. p. 98° $[\alpha]_D$ −20·8°).

3β-*Chlorostigmast-5-ene.*—Diphenyl stigmasteryl phosphate (70 mg.) and hydrochloric–acetic acid similarly afforded a product (40 mg.; m. p. 83—86°), which crystallised from ethanol as plates, m. p. 89—90° (Found: C, 80·8; H, 10·8. Calc. for $C_{29}H_{47}Cl$: C, 80·8; H, 11·0%) (Marker and Lawson, *J. Amer. Chem. Soc.*, 1937, **59**, 2711, give m. p. 83°).

3β-*Chloroandrost-5-en-17-one.*—The crude product (50 mg.; m. p. 128—140°) from dehydro-

*epi*anhydrosterone diphenyl phosphate (100 mg.) and hydrochloric–acetic acid crystallised from methanol, giving colourless plates, m. p. 155—157°, $[\alpha]_D$ +7·7° (Found: C, 73·65; H, 9·1. Calc. for $C_{19}H_{27}OCl$: C, 74·3; H, 8·9%) (Wallis and Fernholz, *J. Amer. Chem. Soc.*, 1937, **59**, 764, give m. p. 154°, $[\alpha]_D$ +14·6°; Butenandt and Grosse, *Ber.*, 1936, **69**, 2776, m. p. 155—157°, $[\alpha]_D$ +14°).

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