PHOSPHONIC ACIDS - X

STEROIDAL PHOSPHONIC ESTERS[1]

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ABSTRACT

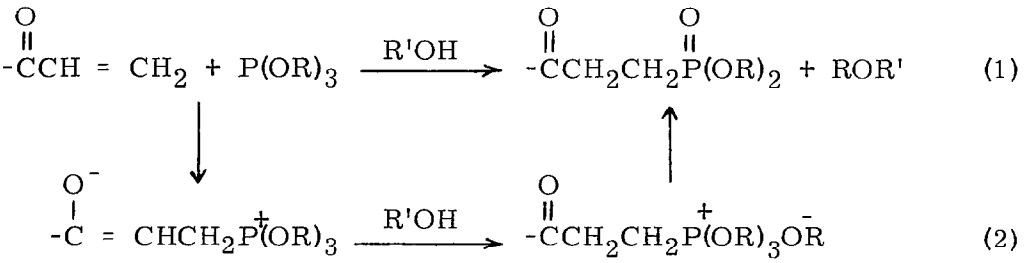
Steroidal phosphonate esters having the dialkoxyphosphinyl group, $(RO)_2P(O)$-, as a ring substituent have been synthesized via reaction of the appropriate α,β-unsaturated ketosteroid with trialkyl phosphite in alcohol. By this means a phosphorus substituent has been introduced into the 1-positions of testosterone, hydrocortisone, and cortisone, into the 7-position of testosterone and into the 16-positions of pregnenolone and progesterone. Monodealkylation of the phosphonate derivatives of the latter two substances was effected by warming with alcoholic sodium propylmercaptide.

Conjugation, long accepted to be involved in the inactivation and excretion of steroid hormones, has recently been recognized to play a broader role: certain conjugates (e.g. dehydroepiandrosterone sulfate and testosterone glucuronide) compete effectively with the free compounds *in vivo*[2,3], and it has been proposed[3] that conjugation may be intimately involved in the secretion and distribution of steroid molecules. On the other hand, the importance of phosphorylated derivatives is uncertain[4,5], and investigation has been hampered by the rapid *in vivo* hydrolysis of administered steroidal phosphate esters[6] due to the ubiquitous phosphomonesterases.

As part of a program to determine the influence of a phosphorus function on biological activity, the synthesis of a series of steroidal phosphonate esters was undertaken. These compounds,

differing from the corresponding phosphate esters by either omission of the oxygen link or its replacement by a methylene unit, were expected to be resistant to removal of the phosphinyl group as a consequence of the hydrolytic stability of the carbon-phosphorus bond[7]. Initial studies in the 11-deoxy series furnished dialkyl and monoalkyl esters of pregnenolone and progesterone 21- and 21a-phosphonic acids[1], and synthesis of similar analogs of the clinically useful corticoid phosphate esters[6] is in progress.

The present paper describes the introduction of the dialkoxyphosphinyl group, $(RO)_2P(O)$-, into the 1, 7 and 16 positions in the steroid ring system via hydrophosphinylation[8,9] of the appropriate α,β-unsaturated ketosteroid with trialkyl phosphite in alcohol or phenol solution (eq. 1). According to the available evidence[9-11], this reaction is ionic in nature and involves attack of the nucleophilic phosphorus reagent on the terminal carbon of the conjugated system, followed by protonation by the solvent and valency expansion of phosphorus[10] to form a P=O bond (eq. 1 and 2).

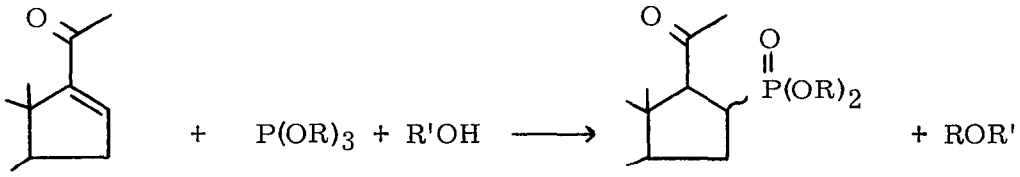


RESULTS AND DISCUSSION

Preliminary tests carried out with the acetate of 3β-hydroxypregna-5,16-dien-20-one (I) (Table 1) demonstrated the feasibility of this transformation and confirmed the previously noted[9,10] superiority of phenol relative to alcohol as a solvent-reactant. Reaction in refluxing isopentyl alcohol (b.p. 131°) or ethanol

TABLE 1

Hydrophosphinylation of 3β-Acetoxypregna-5,16-dien-20-one(I)[a]



I II III

R	R'	Molar ratio (II/I)	Time (hr)	Yield of III (%) [b]
i-C_5H_{11}	i-C_5H_{11}	1	20	29
i-C_5H_{11}	i-C_5H_{11}	2	48	53
Et	Et	1	48	41
i-Pr	i-Pr	1	48	5
Et	Ph	1.5	24	99

[a] Reactions were carried out in an excess of refluxing alcohol, except for reaction in phenol where a temperature of 100° was maintained.

[b] III was obtained in deacetylated form in all except the last example.

(b.p. 78.5°) over a 48 hour period afforded 16-dialkoxyphosphinyl-pregnenolone (III) in 53 and 41% yields, respectively, while a 24 hour reaction in phenol furnished the same phosphonate (as its acetate) virtually quantitatively. Enhanced yield in this latter solvent is probably a consequence of its greater relative acidity.

Treatment of a series of unsaturated ketosteroids with a trialkyl phosphite in phenol at 100° furnished the related 1,7 or 16-dialkoxyphosphinyl derivatives listed in Table 2. It was found necessary to protect reactive alcohol functions (e.g. the 17-OH of testosterone and the 21-OH of cortisone and hydrocortisone) by prior acetylation to prevent their esterification by phosphite. Although a crystalline phosphite ester was previously obtained from a 17α-hydroxypregnenolone derivative[1], those encountered in the present series were viscous syrups, difficult to characterize.

Since 16-dehydroprogesterone undergoes hydrophosphinylation readily, while progesterone remains unaffected under similar conditions[1], reaction of the former substance apparently occurs in the D-ring. Conclusive proof was provided by conversion of III (via chromium trioxide oxidation followed by acid catalyzed isomerization) into 16-diethoxyphosphinylprogesterone (V), identical in every respect to the phosphonate ester from 16-dehydroprogesterone.

The orientation of the phosphorus substituent at the 1,7 and 16-positions is presumed to be α because of the appreciably greater steric and electrostatic non-bonding interactions hindering approach of the bulky phosphorus reagent to the β-face[15]. Although an enolate anion intermediate results from phosphite attack at C-16, β-orientation of the side-chain is thermodynamically favored in the absence of substituents in the 16 position[12,13], and the presence of the 16α-dialkoxyphosphinyl group should reinforce this tendency. Lending further support is the demonstration by Fukushima and Gallagher[14] that Michael-type addition of alkoxide ion to I and to 16-dehydroprogesterone furnished exclusively 16α-alkoxy products. While no conclusion regarding conformation at C-1 may be made on the basis of the NMR data (due to the limited number of analogous phosphonate esters known), it appears that the presence of the 1-dialkoxyphosphinyl substituent is

TABLE 2

Steroid Phosphonate Esters

Steroid	Phosphinyl substituent	m.p. (°C)	UV max (mμ)	ε	Specific rotation	C Calc.	C Found	H Calc.	H Found	P Calc.	P Found
Pregnenolone	16-diethoxy	187.5-188.8	239	66	+18	66.34	66.14	9.13	8.59	6.85	6.78
"	16-diisoamyloxy	157-159	235	252	+17	69.37	68.91	9.95	10.05	5.77	5.85
"	16-ethoxy hydroxy[a]	207.1-208.6				65.07	65.11	8.78	8.47	7.30	7.32
" acetate	16-ethoxy hydroxy[b]	238.5-240				62.85	63.36	8.26	8.52	7.05	6.58
Progesterone	16-diethoxy	159.4-161	240	14,290	+116	66.64	67.13	8.73	9.00	6.88	6.82
"	16-ethoxy hydroxy[a]	214.5-216.5				65.38	64.70	8.35	8.52	7.33	7.38
Testosterone	7-diethoxy[c]	135.5-137.8	241	14,200		65.07	65.53	8.79	8.67	7.30	7.18
" acetate	7-diethoxy	176.5-178	241	15,920	+34	64.36	64.29	8.43	8.29	6.64	6.75
" acetate	1-diethoxy	87-88.3				64.36	64.15	8.43	8.29	6.64	6.63
Cortisone acetate	1-diethoxy	219-221	242	12,710	+154	60.21	60.62	7.30	7.51	5.75	5.75
Hydrocortisone acetate	1-diethoxy	261-263	238	15,780	+70	60.00	60.47	7.64	7.35	5.73	5.96

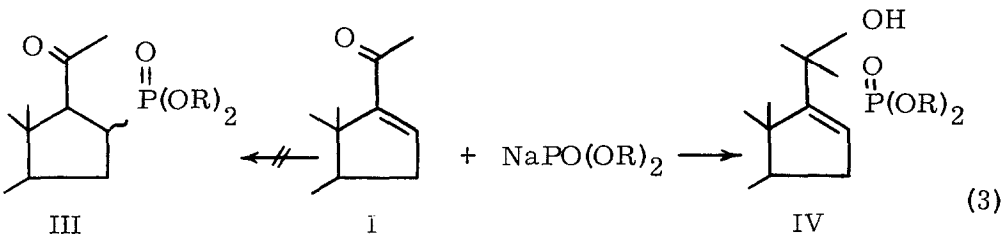
[a] Prepared by monodealkylation of the corresponding diethoxy compound with propyl mercaptide.
[b] Prepared by acetylation of the 3β-ol in refluxing glacial acetic acid for 4 hours.
[c] Prepared by deacetylation of the 17β-acetate in ethanol acidified with conc. HCl.

responsible for a downfield shift of 10-16 cps observed for the C-19 methyl protons. Thus the NMR spectra of the testosterone, hydrocortisone, and cortisone phosphonates exhibited C-19 methyl proton absorptions at τ 8.62, 8.44, and 8.44, respectively; the analogous protons of the parent steroids appeared at τ 8.80, 8.57, and 8.60, respectively.

1-Dehydroprogesterone was transformed by triethyl phosphite in phenol to a product differing from the expected 1-diethoxyphosphinylprogesterone by the incorporation of additional oxygen (2 atoms based upon C, H, P analysis) and the presence of a strong hydroxyl peak (3.02 μ) in the infrared. This compound, which may be a hydroperoxide, was not investigated further, and no analogous product was detected in the testosterone, hydrocortisone or cortisone series.

Two alternative hydrophosphinylation methods were

investigated, but found unsatisfactory. Thus, sodium dialkylphosphonate added exclusively across the carbonyl of I to furnish 3β-acetoxy-20-hydroxy-20-dialkoxyphosphinylpregna-5,16-diene (IV) (eq. 3) in preference to Michael-type addition at C-16 to provide III. Attack on carbonyl carbon by this reagent is reported to be favored by steric hindrance to β-approach[16]; that this is the sole directing factor seems unlikely in view of the apparent accessibility of the C-16 carbon from the α-face of the D-ring. Attempted peroxide-catalyzed addition of diethyl phosphonate to the conjugated double bond of I led to recovery of the unreacted steroid. On the basis of these unpromising results, further investigation of these alternative methods was not pursued.



III I IV (3)

Finally, monodealkylation of the 16-dialkoxyphosphinyl derivatives of progesterone and pregnenolone to the corresponding monobasic phosphonic acids was effected smoothly by warming with alcoholic sodium propylmercaptide[1,17].

EXPERIMENTAL

All reactions with phosphite esters were carried out in a nitrogen atmosphere (to minimize air oxidation). Unless otherwise specified optical rotations were measured in $CHCl_3$, UV spectra in EtOH and IR absorption in solid KBr. M.p.'s were taken on a Leitz Kofler hot-stage microscope and are corrected.

Commercial triethyl phosphite (TEP) and triisopropyl phosphite (TPP) were redistilled and stored under an inert atmosphere; triisopentyl phosphite (b.p. 125-26°/5.5 mm., n_D^{22} 1.4332, reported[18] b.p. 270-75°) was prepared by reaction of isopentyl alcohol with phosphorus trichloride and pyridine in ether at 0-10°.

1-Dehydrotestosterone acetate and 16-dehydroprogesterone were purchased from the Sigma Chemical Co. and the Aldrich Chemical Co., respectively. Prednisone, prednisolone, 6-dehydrotestosterone and 16-dehydropregnenolone acetate were kindly supplied by the Upjohn Co. 1-Dehydroprogesterone (m.p. 152-4°; reported[19] 152-3°) was prepared by dehydrogenation of progesterone with an equimolar quantity of 2,3-dichloro-5,6-dicyanobenzoquinone in refluxing benzene for 3 hrs; after removal of the hydroquinone by filtration, the product was chromatographed on silica gel. Florisil, (100-200 mesh) supplied by the Floridin Co., was activated overnight at 100° prior to use.

Reaction of 3β-Acetoxypregna-5,16-dien-20-one (I) with TEP in Phenol. - A solution of I (12.48 g, 0.35 mole) and TEP (8.81 g, 0.53 mole) in 10 g of phenol was stirred at 100° for 24 hours under nitrogen and chromatographed on Florisil. Successive elution with 1:1 ether-pentane, ether, and acetone-ether furnished phenol and phenetole, recovered I (115 mg), and finally the acetate of III (R=Et) (17.20 g, 99%), respectively.

Mild alcoholysis of the latter in refluxing ethanol containing hydrochloric acid furnished III (R=Et); ν max 2.94 (OH); 5.86 (C=O); 8.16 (P=O); 8.60 (POEt); and 9.72 (POC) μ).

Reaction of I with TEP in Ethanol. - A solution of I (3.56 g, 10 mmole) and TEP (1.66 g, 10 mmole) in 20 ml ethanol was heated at reflux for 48 hours. On cooling, the solution deposited unreacted I. Chromatography of the remainder on Florisil furnished III (R=Et) (2.38 g) still partially in acetylated form (shown by the presence of the ester carbonyl peak in the infrared) as well as an additional quantity of recovered I (430 mg. 12%).

Methanolysis of the latter (2.26 g) in refluxing methanol (15 ml) containing 0.5 ml hydrochloric acid, followed by partition between aqueous sodium carbonate and ether furnished, on evaporation of the ether extracts, III (R=Et) (1.86 g, after recrystallization from ethyl acetate-hexane, 41%).

16-Diisopentyloxyphosphinylpregnenolone (III; R = i-C_5H_{11}). A solution of I (3.56 g, 0.01 mole) and triisopentyl phosphite (2.92 g, 0.01 mole) in 20 ml isopentyl alcohol was maintained at reflux for 20 hours. On cooling, the solution deposited 16-dehydropregnenolone (370 mg, m.p. 205-10°). Chromatography of the remainder on Florisil gave on elution with 5% acetone in ether, III (R = i-C_5H_{11}) (recrystallized from acetone-hexane; ν max 2.88 (OH); 5.82 (C=O); 8.16 (P=O); 8.70 (POEt); and 9.40, 10.14 (POC) μ).

Repetition employing double the quantity of P (III) ester and a reaction period of 48 hours furnished, after evaporation of volatile material and trituration with pentane, III (R = i-C_5H_{11}) (2.16 g, m.p. 154-6°, 40%). Chromatography of the filtrate furnished an additional 682 mg. (13%) of III as well as 16-dehydropregnenolone (606 mg, 17%).

Monodealkylation of III (R = Et). - A solution of the phosphonate (1 g, 20 mmole) in 18 ml n-propyl mercaptan was added to a solution of sodium (460 mg, 20 mmole) in 10 ml ethanol, and the resulting mixture was heated at reflux for 23 hours, then poured into ice water (300 ml) and extracted with ether. Acidification of the ether solution precipitated a white solid which was filtered off, washed with water and dried. The crude monoester (893 mg) was recrystallized from acetone-hexane to provide pure 16-(ethoxyhydroxy)phosphinylpregnenolone (72%).

A solution of the monoethyl ester (500 mg) in 10 ml glacial acetic acid was maintained at reflux for 4 hours, then evaporated in vacuo to furnish the acetate of 16-(ethoxyhydroxy) phosphinylpregnenolone (475 mg, m.p. 232-6° dec.) Recrystallization from acetone-methanol provided the analytical sample.

Reaction of I with TPP in 2-propanol. - Interaction of the reagents, under conditions duplicating those employed for the analogous reaction of TEP, led to an 88% recovery of I and 415 mg of the crude acetate of III (R = i-Pr) (ν max 5.76, 5.84 (C=O); 9.7-10.3 (POC) μ).

Deacetylation of a portion (250 mg) of this ester in 5 ml refluxing methanol containing 5 drops of conc. hydrochloric acid provided III (R = i-Pr) (210 mg. m.p. 131-42°), further purification of which was not successful.

Reaction of I with Sodium Diethyl Phosphonate. - A solution containing sodium methoxide (133 mg) dissolved in a 10 ml THF and 1 ml methanol was added over 15 minutes to a stirred solution of I (7.58 g, 20.8 mmoles) and diethyl phosphonate (2.86 g, 20.8 mmoles) in 40 ml dry THF, then heated at reflux for 30 minutes. The resulting basic, cloudy solution was stored in the cold overnight. Acetone was added, and the insoluble residue was removed by filtration and washed with acetone. Recrystallization of the acetone soluble portion from acetone-cyclohexane furnished 5.59 g of crude phosphonate, recrystallization of which from the same solvent provided the

analytical sample of IV (m.p. 222-23°) whose IR spectrum exhibited a strong OH absorption at 2.96 μ and a single sharp carbonyl band at 5.83 μ as well as peaks at 8.14, 8.79, and 9.70 μ, characteristic of P=O, POEt and POC functions, respectively.

Anal: Calcd. for $C_{27}H_{43}O_6P$: C, 65.57; H, 8.76; P, 6.26. Found: C, 65.38; H, 9.36; P, 5.90.

16-Diethoxyphosphinylprogesterone (V). - A solution of 16-dehydroprogesterone (10.98 g, 35 mmole) and TEP (6.65 g, 40 mmole) in 10 g phenol was stirred at 100° for 24 hours and chromatographed on Florisil. By elution first with 1:1 ether-pentane, then with acetone the mixture was separated into phenol and phenetole (10.44 g) and product-containing fractions. The middle fractions of the acetone-eluate contained V (6.82 g, m.p. 154-57.5°; recrystallization from cyclohexane gave 5.87 g, m.p. 159.4-161° (37%); ν max 5.86, 5.98 (C=O), 6.19 (C=C), 8.02 or 8.12 (P=O), 8.70 (POEt); 9.52, 9.72 (POC) μ). Rechromatography of the early fractions eluted by acetone yielded 16-dehydroprogesterone (629 mg. 5.7%) and V (2.33 g, 14.7%). An additional 4.39 g (27.7%) of V was obtained by rechromatography of the final fractions of the original chromatography; overall yield of V was 79%.

Monodealkylation of V. - n-Propyl mercaptan (53 ml) and V (2.9 g) were added to a solution prepared from 1.21 g of sodium in ethanol (21 ml), and the resulting solution was stirred at reflux temperature for 24 hours, then cooled, poured into ice water (100 ml) and extracted with chloroform. The organic phase was dried over sodium sulfate, evaporated to dryness, and the solid obtained was adsorbed on a column of Dowex 50W-X8 resin and eluted with 50% aqueous ethanol. Trituration of the intermediate fractions (2.08 g) with acetone furnished 16-(ethoxyhydroxy) phosphinylprogesterone (1.61 g).

7-Diethoxyphosphinyltestosterone. - The product obtained from interaction of 6-dehydrotestosterone acetate (4.8 g, 14.6 mmole) and TEP (4.99 g, 30 mmole) in phenol (15 g) under standard conditions was freed of phenol and phenetole by chromatography on Florisil, then recrystallized from acetone-hexane to provide 17β-acetoxy-7-diethoxyphosphinyltestosterone (presumably as the α-epimer) (3.21 g; ν max 5.78, 5.97 (C=O); 6.21 (C=C); 8.08 (P=O) and 9.50, 9.72 (POC)μ).

Deacetylation by warming 3.06 g of the phosphonate in acidic (0.15 ml conc. HCl) ethanol (10 ml.) for 3.5 hours gave a crude product separated by chromatography on Florisil into the acetate (374 mg.) and free phosphonate (2.35 g). Recrystallization of the latter from acetone-hexane gave 7-diethoxyphosphinyltestosterone (1.57 g, ν max 2.95 (OH); 5.98 (C=O); 6.20 (C=C); 8.09 (P=O) and 9.60, 9.74 (POC)μ).

17β-Acetoxy-1-diethoxyphosphinyltestosterone. - Hydrophosphinylation of 1-dehydrotestosterone-17-acetate (4.80 g, 14.6 mmole) under similar conditions to those employed for the 7-isomer furnished 17β-acetoxy-1-diethoxyphosphinyltestosterone (5.60 g, recrystallized from acetone-hexane; ν max 5.78, 5.97 (C=O); 8.05 (P=O); 9.50, 9.80 (POC)μ).

21-Acetoxy-1-diethoxyphosphinylcortisone. - Treatment of prednisone-21-acetate (5 g, 12.4 mmole) with TEP (3.1 g, 18.6 mmole) in phenol (8 g) at 100° for 24 hours followed by chromatography on Florisil provided 21-acetoxy-1-diethoxyphosphinylcortisone (5.40 g) as a solid, as well as 1-diethoxyphosphinylcortisone (984 mg) as a resin. Recrystallization of the major product twice from benzene-cyclohexane and twice from acetone-hexane provided the analytical sample (3.53 g, ν max 3.02 (OH); 5.72, 5.81, 5.88, 5.98 (C=O); 6.23 (C=C); 7.9-8.2 (P=O obscured by interfering absorptions); 8.70 (POEt); 9.56, 9.78 (POC)μ).

21-Acetoxy-1-diethoxyphosphinylhydrocortisone. - Hydrophosphinylation of prednisolone-21-acetate under similar conditions to those employed for its 11-keto analog provided the expected phosphonate (ν max 2.90 (OH); 5.73, 5.81, 6.02 (C=O); 6.22 (C=C); 8.08 (P=O); 8.77 (POEt); 9.62, 9.78 (POC)μ).

Reaction of 1-Dehydroprogesterone with TEP. - The product mixture from reaction of 1-dehydroprogesterone (1.1 g, 3.5 mmole) with TEP (5.3 mmole) in phenol (2 g) at 100° for 24 hours was chromatographed on Florisil. The crude phosphonate (1.16 g) was recrystallized from benzene-cyclohexane and from acetone-hexane to furnish a solid (412 mg, m.p. 202-5°; ν max 3.02 (OH); 5.84, 5.95 (C=O); 6.20 (C=C); 8.10 (P=O); 8.74 (POEt); 9.47, 9.82 (POC)μ).

Anal: Calcd. for $C_{25}H_{39}O_7P$. C, 62.22; H, 8.15; P, 6.42. Found: C, 62.18; H 8.06; P, 6.45.

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