Solvent was removed to leave 8 mg of a crude solid, which was purified by column chromatography over 5 g of silica gel, eluting with 60% ether in hexanes, to afford 6 mg (43%) of **6** identical in all respects with material obtained by rearrangement of **2**.

Bicyclo[5.2.1]decane-4,10-dione 4-Ethylene Ketal (17). A solution of 0.641 (4.1 mmol) of keto ketal **15** in 15 mL of dry methanol was stirred under dry nitrogen at ice bath temperature with 0.134 g (0.97 mmol) of powdered anhydrous K_2CO_3. A solution of 1.183 g (4.1 mmol) of dinitrosocarbamate **16** in 20 mL of CH_2Cl_2 was added dropwise over 1 h and the resulting solution stirred for 3 h, during which time it warmed to room temperature. The reaction mixture was poured into 150 mL of water and 50 mL of brine and extracted with three 50-mL portions of CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 50 mL of aqueous Na_2CO_3 and 50 mL of brine, dried over $MgSO_4$, and evaporated to leave 0.884 g of crude product which was chromatographed over 70 g of silica, eluting with hexane–ether (5:2), to give 0.251 g (34%) of an oil: ^1H NMR ($CDCl_3$) δ 1.4–2.5 (14 H), 3.8 (4 H, s); ^{13}C NMR ($CDCl_3$) δ 24.8, 27.1, 33.8, 44.7, 63.2, 64.4; IR (CCl_4) 1735 cm^{-1}; mass spectrum, 210 (0.17), 182 (5.68), 99 (10.59), 55 (11.61); high-resolution mass spectrum calcd for $C_{12}H_{18}O_3$ m/e 210.1255, obsd m/e 210.1249.

1-Methylbicyclo[5.2.1]decane-4,10-dione (18). Method I. A solution of 131 mg (0.74 mmol) of unsaturated bicyclic diketone **7** in 13 mL of 95% ethanol was hydrogenated at atmospheric pressure over 20 mg of 10% Pd on carbon. When 1 equiv of hydrogen had been absorbed, the solution was filtered to remove the catalyst, and the solvent was removed at reduced pressure to leave 134 mg of crude product which was chromatographed over 8 g of silica, eluting with hexane–ether (2:1), to afford 95 mg of pure saturated diketone.

Method II. To a solution of 0.13 mL (94 mg, 0.93 mmol) of diisopropylamine in 10 mL of dry DME at ice bath temperature was added dropwise over 15 min 0.7 mL (0.97 mmol) of a 1.38 M solution of *n*-BuLi in hexane. After the mixture was stirred for a further 10 min, 184 mg (0.87 mmol) of ketal ketone **17** in 7 mL of DME was added dropwise over 30 min, and stirring was continued for an additional 30 min before addition of 1 mL (16 mmol) of methyl iodide. After 90 min the reaction mixture was poured into 300 mL of 5% aqueous HCl before extraction with three 100-mL portions of ether. The combined ether extracts were washed with 100 mL of 5% aqueous HCl and 100 mL of brine and dried over $MgSO_4$. Removal of solvent at reduced pressure left 73 mg of crude product which was chromatographed over 9 g of silica, eluting with hexane–ether (3:2), to afford 31 mg of the title compound.

Both samples gave identical spectral data: ^1H NMR ($CDCl_3$) δ 1.05 (3 H, s), 1.5–3.0 (13 H); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.4, 29.1, 34.0, 36.2, 38.0, 39.7, 45.3; IR (CCl_4) 1740, 1710 cm^{-1}; mass spectrum, 180 (0.75), 162 (0.66), 152 (1.25), 110 (2.51), 97 (5.63), 55 (6.34).

Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.01; H, 8.96.

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Registry No. **1**, 75400-29-2; **2**, 75400-30-5; **3**, 75400-31-6; **4**, 7424-82-0; **5**, 75400-32-7; **6**, 75400-33-8; **7**, 75400-34-9; **8**, 75400-35-0; **10a**, 75400-36-1; **10b**, 75400-37-2; **11a**, 75400-38-3; **11a** xanthate, 75400-39-4; **12**, 75400-40-7; **13**, 75400-41-8; **14**, 75400-42-9; **15**, 4746-97-8; **16**, 19935-89-8; **17**, 75400-43-0; **18**, 75400-44-1; 2-methylcyclopentanone, 1120-72-5; methyl acrylate, 96-33-3; methyl 3-bromo-1-methyl-2-oxocyclopentanepropanoate, 75400-45-2; 1-methyl-2-oxo-3-cyclopentenepropanoic acid, 75400-46-3; vinyl bromide, 593-60-2; 1β-(benzoyloxy)-8aβ-methyl-1,2,3,3aβ,4,7,8-heptahydro-6(7*H*)-azulenone 6-ethylene ketal, 75400-47-4; 8a-methyl-3,3a,4,5,8-pentahydro-2-(trimethylsilyloxy)-1,6(2*H*,7*H*)-azulenedione 6-ethylene ketal, 75400-48-5.

Reactions of Steroid Salts with Hexachlorocyclotriphosphazene[1]

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The sodium salts of the steroids desoxoestrone (VI), estrone (VII), β-estradiol (VIII), 17α-ethynylestradiol (IX), estradiol 3-methyl ether (XIV), and 1,4-dihydroestradiol 3-methyl ether (XV) were found to react with hexachlorocyclotriphosphazene (III) to yield species of the general formula $N_3P_3Cl_5(OR)$ (IV). The remaining halogen atoms were replaced by methylamine to yield hydrolytically stable derivatives of formula $N_3P_3(NHCH_3)_5(OR)$ (V). These are model compounds for the analogous steroid-substituted phosphazene linear high polymers. Aliphatic steroids such as cholesterol (X), dihydrocholesterol (XI), pregnenolone (XII), estradiol 3-methyl ether (XIV), or 1,4-dihydroestradiol 3-methyl ether (XV) underwent dehydration as well as substitution in contact with III. Mestranol (XVII) as its 17-sodium salt did not react rapidly with III, presumably for steric reasons. At elevated temperatures, estrone 3-methyl ether (XVI) interacted with III by a complex process that involved elimination of hydrogen chloride. All the chlorine atoms in III could be replaced by a reaction with the sodium aryloxide salt of estrone (VII). The replacement followed a nongeminal pathway. The significance of these results for the synthesis of the related high polymers is discussed.

Considerable interest exists in the synthesis of high molecular weight polymers that may function as carrier molecules for chemotherapeutic agents.[2-8] Phosphazene high polymers with steroidal side groups could be potentially useful drugs or prodrugs, assuming that a slow hydrolytic release of the steroid could occur in vivo. Moreover, the possibility exists that steroidal phosphates might be released during hydrolysis, species that have themselves been used in steroid therapy.[9-11] However, effective

(1) For a previous paper in this series, see P. P. Greigger and H. R. Allcock, *J. Am. Chem. Soc.*, **101**, 606 (1979).
(2) L. G. Donaruma in "Synthetic Biologically Active Polymers", A. D. Jenkins, Ed., Pergamon Press, New York, 1975, Chapter 1.
(3) H.-G. Batz, *Adv. Polym. Sci.*, **26**, 25 (1977).
(4) L. G. Donaruma and O. Vogl, Ed., "Polymeric Drugs", Academic Press, New York, 1978.
(5) A. Zaffaroni and P. Bonsen, ref 4, p 1.
(6) H. R. Allcock, R. W. Allen, and J. P. O'Brien, *J. Chem. Soc., Chem. Commun.*, 717 (1976).
(7) R. W. Allen, J. P. O'Brien, and H. R. Allcock, *J. Am. Chem. Soc.*, **99**, 3984 (1977).
(8) H. R. Allcock, R. W. Allen, and J. P. O'Brien, *J. Am. Chem. Soc.*, **99**, 3987 (1977).
(9) R. J. W. Cremlyn and I. Khattak, *Phosphorus*, **6**, 237 (1976).
(10) R. B. Brownfield and W. Schultz, *Steroids*, **2**, 597 (1963).

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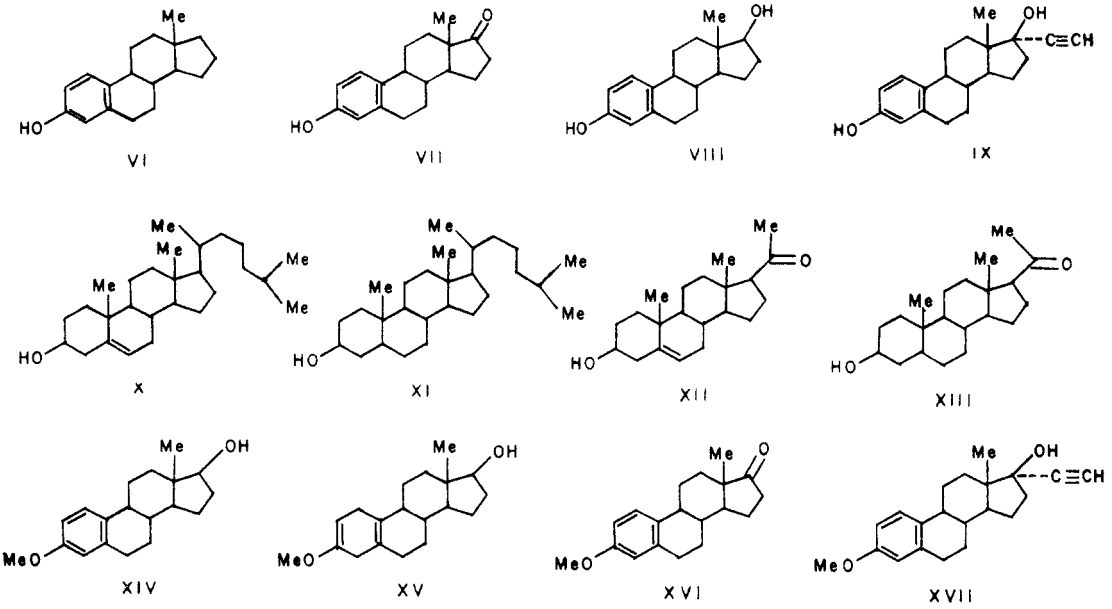
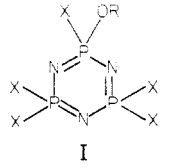
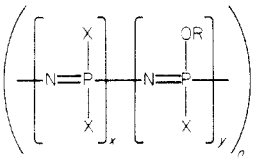
Chart I



macromolecular syntheses and reactivity studies usually require prior studies with small-molecule analogues. Cyclic trimeric phosphazenes are useful models for the linear high polymers,[14] and in this paper we discuss a series of model compound studies with steroidal-substituted cyclic trimeric phosphazenes.

The compounds studied in this work have the general structure shown in I, where OR is a steroidal residue bound

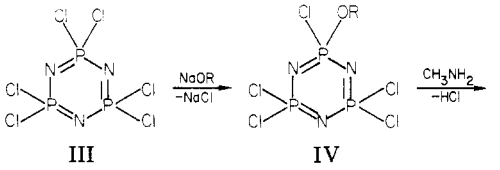

I

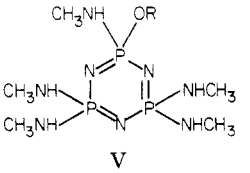

II

to a phosphazene skeleton via the 3- or 17-position. These species serve as models for high polymers of structure II. In principle, the group X can be a cosubstituent unit designed to solubilize the molecule in water ($NHCH_3$) or destabilize it to hydrolytic attack ($NHCH_2COOC_2H_5$,[15] imidazolyl,[16] or NH_2[17]). In this paper, we restrict our attention exclusively to the use of the methylamino group as a cosubstituent species, although it is recognized that the use of amino acid ester or imidazolyl groups could provide hydrolysis products which are more biocompatible.

From a fundamental point of view, the attachment of steroidal residues to a phosphazene ring offers the prospect of mechanistic comparisons with the less complex alkoxy- and (aryloxy)phosphazenes that have already been investigated in detail.[18] For example, the reactions of hexachlorocyclotriphosphazene, $(NPCl_2)_3$, with alkoxides and aryl oxides are normally rapid and complete. However, evidence exists[19,20] that bulky nucleophiles generate steric hindrance effects, both with respect to the introduction of the nucleophile itself and to the subsequent introduction of other side groups. Nucleophiles based on a steroid framework offer a means for an exploration of such steric effects.

The specific reaction sequence discussed in this paper is the conversion of III to IV and V. The alternative


III IV


V

sequence—an introduction of the methylamino residues first, followed by the steroid residue—was not attempted, because of the known high reactivity of methylamine toward III and the probability of complete chlorine replacement by the amine.

The principal steroids used in this work were desoxoestrone (VI), estrone (VII), 17β-estradiol (VIII), 17α-ethynylestradiol (IX), and estradiol 3-methyl ether (XIV) (see Chart I). However, a number of other steroids were also examined in an attempt to explore the scope of these reactions. These included cholesterol (X), dihydrocholesterol (XI), pregnenolone (XII), 20-oxo-5α-pregnan-3β-ol (XIII), 1,4-dihydroestradiol 3-methyl ether (XV), estrone 3-methyl ether (XVI), and mestranol (XVII).

These steroids were chosen for the following reasons. The steroidal residue from VI (itself derived from VII) represented the simplest steroid structure that extended

(11) The phosphate group has also been used to facilitate the administration of aqueous solutions of corticosteroid esters without reduction of physiological activity.[9,12,13]
(12) T. Reichstein and W. Schlindler, *Helv. Chim. Acta*, **23**, 669 (1940).
(13) S. Harai, R. G. Harvey, and E. V. Jensen, *Tetrahedron*, **22**, 1625 (1966).
(14) H. R. Allcock, *Acc. Chem. Res.*, **12**, 351 (1979).
(15) H. R. Allcock, T. J. Fuller, D. P. Mack, K. Matsumura, and K. M. Smeltz, *Macromolecules*, **10**, 824 (1977).
(16) H. R. Allcock, T. J. Fuller, K. Matsumura, and D. Mantz, unpublished work.
(17) D. B. Sowerby and L. F. Audrieth, *Chem. Ber.*, **94**, 2670 (1961).
(18) H. R. Allcock, "Phosphorus–Nitrogen Compounds", Academic Press, New York, 1972.
(19) H. Schadow and H. Scheler, *Z. Chem.*, **13**, 223 (1973).
(20) H. R. Allcock, S. D. Wright, and K. M. Kosydar, *Macromolecules*, 11, 357 (1978).

the known reactions of phenoxide ion[18] with III. As such, it formed a primary model against which the other steroid structures could be compared. The structures derived from VII and XII provided a test for the stability of keto or acetyl functions under the chosen reaction conditions. Furthermore, VII–IX, XII, and XIII should be pharmacologically active in the covalently bound form (V). Compounds X–XIII also constituted examples of aliphatic 3-hydroxy functional groups that could be compared with the known reactions of simple alkoxides with III. Steroid XIV was used to explore the reactivity of a secondary alcohol function at the 17-position and to monitor the role played by the angular 18-methyl group. Steroid XVII was utilized both as an example of a species with a tertiary alcohol function at the 17-position and because it is active as a birth control agent. Compound XVII or XIV was used as a control to model the reactivities (a) of the 17-position, (b) of aliphatic alcohols, and (c) of VIII or IX, respectively. Cholesterol (X) provided a control for monitoring the stability of the acetyl group at position 20 in XII or XIII under the reaction conditions employed. Species XI or XIII was employed as a control to deduce the behavior of the double bond in X or XII. Three of the steroids—desoxoestrone (VI), cholesterol (X), and estradiol 3-methyl ether (XIV)—were chosen because they possess only one reactive group and, thus, were not expected to participate in ring-coupling reactions. The C-3 methyl ether groups of the steroids XIV–XVII were expected to block reactions at these sites. Compounds VI–IX offered the prospect that their reactions could be monitored by electronic spectroscopy. Model nucleophiles for steroids VI–IX were provided by phenol and sodium phenoxide and for VII, XII, and XIII by cyclohexanone or XVI and their sodium salts.

With these facts in mind, this study was carried out in an attempt to answer the following questions. (1) Can bulky steroid molecules be attached through the 3- or 17-hydroxy positions to a cyclophosphazene ring to yield species such as IV or V, and what are the preferred experimental conditions needed to achieve that end? (2) Does the introduction of the steroidal residue sterically inhibit the introduction of a second substituent group such as methylamino? (3) Are the steroidal residues in species such as IV stable to subsequent reactions needed to introduce methylamino residues, and is the phosphazene skeleton stable to reactions required to remove protecting groups from the steroid? (4) Might free steroids such as X–XV or XVII undergo dehydration rather than substitution in the presence of $(NPCl_2)_3$?[21] (5) Can phosphazenes derived from X–XIII undergo a migration of alkyl groups from oxygen to skeletal nitrogen in a manner reminiscent of the known behavior of simple alkoxycyclophosphazene?[22]

Results and Discussion

Formation of Steroid Salts. The steroids were converted to their sodium salts by treatment with sodium hydride either at room temperature or in boiling tetrahydrofuran (THF).

Because steroids, such as VII–IX, XII, XIII, and XVII, contain additional active groups that could react with sodium hydride, considerable care was taken to ensure that the reaction of the hydroxyl group was the only process that occurred. For example, estrone (VII) was allowed to react with sodium hydride under mild conditions (see Experimental Section) in very dilute solution to discourage self-condensation reactions. The 17-carbonyl group was virtually unaffected under these conditions. A more detailed study entailed the interaction of XVI with sodium hydride under more forcing conditions, followed by exposure to $(NPCl_2)_3$. No detectable quantity of phosphazene enol was identified, and essentially all of XVI was recovered unchanged.

The ease of sodium salt formation during the reaction of the steroids VI–XVII with sodium hydride was dependent on the relative acidities of the alcohols. Aromatic (3-position) alcohols such as VI–IX reacted immediately with sodium hydride at 25 °C. However, no detectable reactions between the steroids X–XIV, XVI, or XVII with sodium hydride and III were evident (with the use of ^{31}P NMR analysis) even after 24 h at 25 °C. Therefore, it was possible to generate the monosodium salts selectively at the C-3 positions of the steroids VII–IX (at 25 °C) without affecting the other functional sites.

Steroids such as X and XIV reacted with sodium hydride after prolonged boiling in THF (>12 h). It was even possible to prepare the salts of the steroids X–XIV with sodium hydride in the presence of III at 25 °C but at a much slower rate than at the higher temperature. The C-17 alcohol and C-20 alcohol or keto groups of the steroids VII–IX and XII–XVII were unreactive with III even after 24 h in the presence of sodium hydride (see Experimental Section). However, the sodium salts of the C-3 aromatic alcohols of VI–IX reacted rapidly under these same reaction conditions. Thus, multifunctional steroids such as VII–IX reacted with III selectively at C-3 to form IV without the use of protecting groups. It should be noted that these reactions were carried out with *dilute* solutions of the reagents, usually at 25 °C. In concentrated solutions, and especially at higher temperatures ($\simeq$70 °C), side reactions became serious (see the following sections).

Two different procedures were followed to generate salts at the 17-position of mestranol (XVII) without reaction of the acetylenic unit. First, reaction of XVII with excess sodium hydride, followed by removal of the excess sodium hydride, and addition of a stoichiometric equivalent of XVII to the reaction mixture ensured the absence of the acetylide salt. Second, a quantitative addition of methyllithium to XVII yielded the lithium alcoholate.

Reactions of Steroid Salts with $(NPCl_2)_3$. General Features. The reactivities of the alcohol groups or sodium salts of steroids VI–XVII with $(NPCl_2)_3$ were monitored by ^{31}P NMR methods. Treatment of excess III with the sodium salts of VI–IX or XIV in dilute solutions resulted in the rapid formation of monosteroidal-substituted phosphazenes of type IV. Sodium chloride precipitated. The more acidic steroidal alcohols (such as VI–IX) gave higher yields of IV, a result that was ascribed to the relative efficiency of the steroidal salt formation rather than to a direct steric influence during (aryloxy)phosphazene formation.

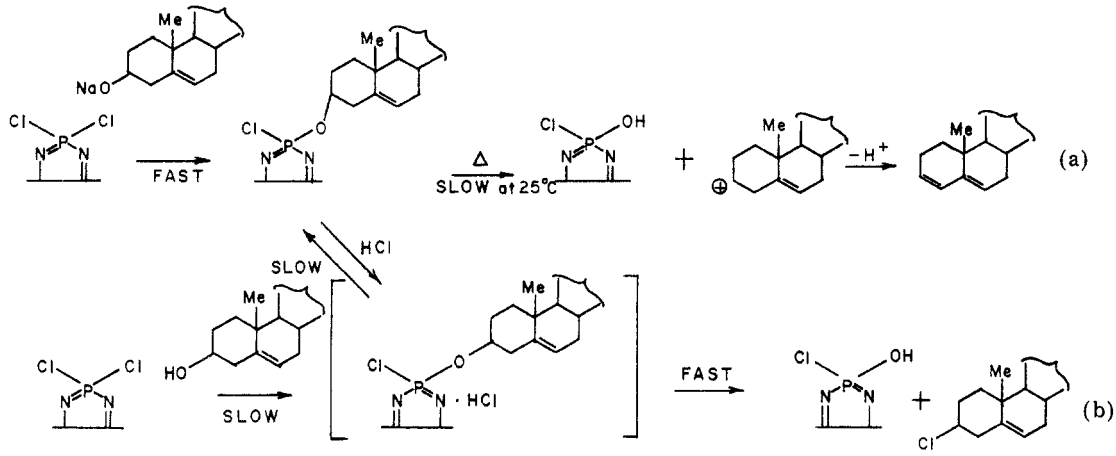
In contrast to the behavior of the sodium salts, the reactions of the alcohol groups of steroids VI–XVII with III were a very slow processes and required long reaction times before a detectable formation of IV resulted.

No products of type IV or V were isolated when the steroidal reagent was derived from XVI or XVII, and only low yields of IV were detected with X–XIII.

Because of their hydrolytic instability, the products of structure IV derived from desoxoestrone (VI), estrone (VII), β-estradiol (VIII), α-ethynylestradiol (IX), cholesterol (X), dihydrocholesterol (XI), and estradiol 3-methyl

(21) M. Kajiwara and H. Saito, *Kogyo Kagaku Zasshi*, 74, 619 (1971); *Chem. Abstr.*, 75, 34923 (1971).
(22) B. W. Fitzsimmons, C. Hewlett, and R. A. Shaw, *J. Chem. Soc.*, 4459 (1964).

Scheme I



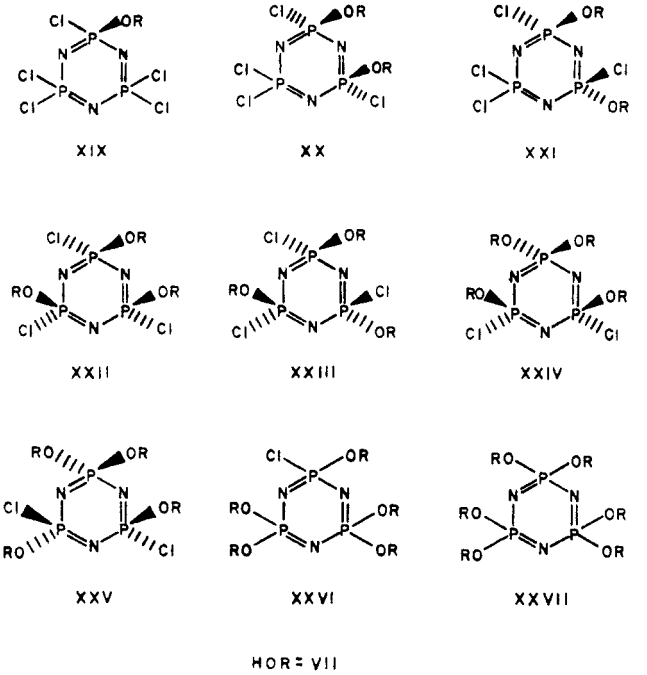
ether (XIV) were not isolated in an ultrapure state. They were characterized in solution by ^{31}P and ^{13}C NMR, infrared, or electronic spectral techniques and were used directly for conversion to V.

Reaction of Estrone with III. Estrone (VII; as its sodium aryloxide salt) reacted with III by a nongeminal substitution pathway to form species XIX–XXVII (Chart II). The low solubility of the sodium salt of VII and its steric bulk affected the rate and the number of estrone groups attached to III. Compounds XIX–XXI were formed at 25 °C (within 12 h), whereas the formations of XXII–XXV required longer reaction times (24 h) under the same experimental conditions. Species XXVI and XXVII were generated only with the use of more forcing conditions. It is surprising that isomers XXII and XXIII were formed in equal amounts. Similarly, the yields of isomers XXIV and XXV were approximately the same. Although the presence of a steroid residue on III induces a nongeminal substitution pathway, it has no apparent influence on the stereochemistry of the steroid attachement to the other phosphorus atoms.

Special Case of Pregnenolone (XII) and Cholesterol (X). Very low yields (<5%) of IV were obtained when the sodium alkoxide salts from X or XII were allowed to react with $(NPCl_2)_3$ by method A (see Experimental Section). This was not a consequence of a low reactivity of the nucleophiles because ^{31}P NMR spectra showed that 17% of the $(NPCl_2)_3$ had reacted with the sodium salt of XII and that 23% of the $(NPCl_2)_3$ had reacted with the salt formed from X. A contributing side reaction appeared to be a dehydration process similar to the one already known for the interaction of cholesterol with phosphorus oxychloride.[23] The mechanism of this dehydration probably involves the intermediate formation of IV, followed by the loss of the phosphazene as a leaving group. This step would be encouraged by anchimeric assistance by the π electrons of the 5,6 double bond to yield a stabilized cation, as shown in Scheme I, pathway a. In fact, mass spectrometric analysis of the reaction products formed from the cholesteryl salt revealed the presence of $C_{27}H_{44}$ ion at m/e 368, and this was assigned to the ion from 3,5-cholestadiene. An ultraviolet absorbance at 234 nm (in hexane) was also consistent with the formation of 3,5-cholestadiene.

When cholesterol (X), III, and sodium hydride were combined (see method B, Experimental Section) and allowed to react, the products were IV (where HOR = X), cholesteryl chloride, and hydroxyphosphazenes. The

Chart II



product IV (OR = residue from X) was formed in approximately 20% yield as detected by ^{31}P NMR methods. Cholesteryl chloride was formed by the reaction between IV and the hydrochloric acid that was liberated by a reaction of the alcohol X with III. Compounds XI–XIII behaved similarly. Cholesteryl chloride was isolated by chromatography and was identified on the basis of its melting point (90–97 °C) and its mass spectrum (parent peak at 402 amu). The reaction pathways for these transformations are included in Scheme I, pathway b.[24]

Other Side Reactions. The ease with which the steroids (as alcohols) were dehydrated by III increased in the following order: XIV = XV < XI ≃ XIII < X. This order parallels the expected ease of carbonium ion formation. Although the alcohol or the sodium or lithium alkoxide salts at the 17-position of mestranol (XVII) failed to react with III at 25 °C in tetrahydrofuran, at elevated temperatures (70 °C), the tertiary alkoxide or alcohol was dehydrated. Similar reactions appeared to take place with

(23) R. J. W. Cremlyn, B. B. Dewhurst, and I. Khattak, *Phosphorus*, 6, 201 (1976).

(24) A third side reaction was also possible but has not been confirmed. The steroid alkyl group could have migrated from oxygen to nitrogen. This reaction is known for the system: $[NP(OR)_2]_{3or4} \rightarrow [N(R)P(O)(OR)]_{3or4}$, where R = methyl, ethyl, propyl, or isopropyl.[22]

estradiol 3-methyl ether (XIV) or with 1,4-dihydroestradiol 3-methyl ether at 70 °C. Even *tert*-butyl alcohol or sodium *tert*-butoxide was converted to isobutylene by III in boiling tetrahydrofuran. No dehydration was detected when the sodium salt of XIV reacted with III to form IV. Side reactions also occurred when estrone 3-methyl ether (XVI) interacted with III. Again, no reaction was evident at 25 °C, but hydrogen chloride was evolved at higher temperatures. (The reactions of compounds XIV and XVII are models for steroids VIII and IX. Thus, under mild experimental conditions, the C-17 alcohols of VIII and IX appear to be unreactive with III.) Dry cyclohexanone behaved similarly, and this suggested that the reaction involved the carbonyl group at position 17 in XVI. The reaction with cyclohexanone was complex. ^{13}C NMR spectra of the reaction mixture showed evidence for the formation of imines (at 175 ppm), C–Cl bonds (68 ppm), and alkenes. The hydrogen chloride liberated caused cleavage of the tetrahydrofuran solvent and condensation of the remaining cyclohexanone. These reactions probably mimic those that occur with XVI. Because XVI is a model for estrone (VII), it is clear that forcing reaction conditions should not be employed for the interaction of estrone with $(NPCl_2)_3$.

Reactions of IV with Methylamine. Methylamine was chosen as a nucleophile for the removal of the remaining halogen atoms in $N_3P_3Cl_5(OR)$ (IV) for the following reasons. First, the methylamino residue is a water-solubilizing side group for cyclo- or polyphosphazenes,[25] and, second, the small steric size of this nucleophile would be expected to provide a maximum impetus for total removal of the remaining chlorine atoms.

In fact, only mild reaction conditions were needed to convert compounds of type IV (where OR = residues from VI–IX or XIV–XV) to species V by treatment with methylamine (see Experimental Section). However, the reaction of the estrone (VII) derivative, $N_3P_3Cl_5OR$, with methylamine yielded first a derivative of type V in which the carbonyl group at the 17-position had been converted to a methylimino residue. Subsequent treatment of this compound with water regenerated the carbonyl group without modification of the remainder of the molecule. The facile replacement of all the chlorine atoms in IV by methylamino groups suggested that similar reactions with the analogous high polymers might be feasible if no more than one steroidal residue was present for every three repeating units. The structural proof for species of type IV or V is described in the Experimental Section.

Solubility and Hydrolytic Stability. Species of type IV and V were soluble in a variety of organic media. Compounds of structure V (HOR = VII–IX) were slightly soluble in water. This implies that the synthesis of water-soluble high polymeric analogues of V would require the presence of fewer than one steroidal residue per three repeating units, except when residues from VII–IX were present. Although the derivatives of structure IV were insoluble in water, they hydrolyzed on contact with atmospheric moisture or aqueous base. Species of type V appeared to be stable to water at 25 °C.

Relationship to Synthesis of Macromolecules. From these results it appears that the analogous high-polymer-synthesis reactions would be extremely complex if pregnenolone, cholesterol, or mestranol were employed as substituent groups. Traces of chain-coupling side reactions would be sufficient to cause total insolubilization of a macromolecular system. However, monofunctional steroids such as VI or XIV or multifunctional steroids such as VII–IX that can be selectively derivatized at one position are suitable for use in a polymer synthesis sequence. Steroids X–XII appear to be unsuitable for this purpose. The facile replacement of the residual halogen in IV by methylamino implies that similar reactions may be possible with the macromolecular species. These prospects will be examined in a future paper.

Experimental Section

Reagents and Solvents. Anhydrous methylamine (Linde Air Products or Matheson) was used as received. Triethylamine (Eastman) was distilled from barium oxide before use. Benzene and heptane (Fisher) were boiled at reflux and were distilled from calcium hydride before use. Tetrahydrofuran (THF) (Fisher) was treated with lithium aluminum hydride or sodium/benzophenone. Sodium hydride, as a 50% dispersion in oil (Alfa Chemical), was washed with freshly distilled heptane before use. Estradiol 3-methyl ether, estrone-3 methyl ether, mestranol, β-estradiol, α-ethynylestradiol, pregnenolone, allopregnanolone,[30] cholesterol or dihydrocholesterol (all from Sigma), and estrone (Sigma or Aldrich) were dried in vacuo for 8 h before use. 1,4-Dihydroestradiol 3-methyl ether was prepared by the method reported by Wilds and Nelson.[31] Hexachlorocyclotriphosphazene (mp 112 °C) was obtained from a cyclic trimer–tetramer mixture (El Monte Chemical Corp. or Ethyl Corp.) after two vacuum sublimations at 60 °C (0.5 torr), two recrystallizations from heptane, and two subsequent sublimations.

Instrumentation. ^1H-Decoupled ^{31}P NMR spectra were obtained at 40.5 Hz with the use of a JEOL PS-100 spectrometer operated in the Fourier transform mode and interfaced with a Nicolet 1080 data processor. Ultraviolet spectra were obtained with Varian 634 or Cary 17 ultraviolet spectrophotometers. Infrared spectra were obtained with the use of Perkin-Elmer Model 267, 261, and 580 spectrophotometers. Mass spectra were obtained with the use of an AEI/MS 902 mass spectrometer operated at an ionization potential of 70 eV. Perfluorinated kerosene was used as an internal reference.

Elemental analyses were obtained by Galbraith Laboratories. The samples were dried in vacuo for 8 h at 40 or 80 °C before analysis.

Synthesis of $N_3P_3(NHCH_3)_5(OCH_2CF_3)$. Methylamine gas (200 mL) was condensed into a reaction vessel (50-mL capacity) equipped with a nitrogen inlet, a rubber septum, and a dry ice condenser which recycled the methylamine gas and maintained the liquid at its boiling point (–6.3 °C). A sample of $N_3P_3Cl_5(OCH_2CF_3)$[26] (10 mL) was injected through the rubber septum by means of a hypodermic syringe and was stirred with the liquid methylamine for 4 h. Unreacted methylamine gas was allowed to escape through a silicone oil bubbler, and the THF-soluble residue was filtered to remove the undissolved methylamine hydrochloride salt. The solvent was allowed to evaporate from the filtrate to leave a white, waxy solid that was washed with hot methylene chloride, was isolated by filtration, and was dried in air to give $N_3P_3(NHCH_3)_5(OCH_2CF_3)$, mp 148–150 °C. The overall yield was close to 100% as deduced by ^{31}P NMR spectra of the reaction mixture (see Table I). A mass spectrum showed a parent ion at m/e 383 (theory m/e 384). An infrared spectrum showed a $\nu_{P=N}$ absorption at 1195 cm^{-1}.

Preparation of Desoxoestrone (VI). This compound was prepared by the conventional method from estrone, with the use of hydrazine hydrate in triethylene glycol. After several recrystallizations of the product from a dilute solution in ethanol

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Table I. Conditions for the Preparation of Steroidal Cyclotriphosphazenes

steroid (amt, mol × 10^3)	amt $(NPCl_2)_3$, mol × 10^3	amt THF, mL	reaction time, h (temp, °C)	mL of methylamine	isolation technique	mp, °C	product type (yield, %)	mass spectral parent ion (theory), m/e
VI (1.2)[a]	1.4	450	12 (65) + 72 (25)		b	c	IV (30)[d,u]	567 (568)
VI (1.43)[e]	1.43	100	24 (65)	100[f]	g, h	142[i]	V (39)[v]	540 (540)
VII[j] (3.7)	3.7	400	12 (25)		k		IV (85)[d]	
VII (12)	2.9	400	12 (25)		k		IV (60)[d]	
VII (17.4)[l]	1.44	350	12 (25)		k		XIX-XXIII[m]	
			24 (25)		k		XXII-XXV[n]	
			4 (65) + 12 (25)		k		XXVI, XXVII[o]	
			16 (65)		k		XXVI, XXV[p]	
			24 (65)		q	270 dec[i]	XXVII (15)	
VII (11)	14.8	125	12 (25)	300[f]	r	155[i]	V (20)	554 (554)
XIV (7)[q]	10.6	250	12 (27)		b		IV (15)[w]	597 (598)
				300[f]	b, r	152-154[i]	V (12.5)	570 (570)
VIII (3.7)[s]	3.7	400	12 (25)				IV (65)[d]	
				400[f]	20	100-105 dec[i]	V (39)	556 (556)
IX (3.4)[t]	3.7	400				100 dec[i]	V (30)	580 (580)

[a] The sodium salt of the steroid was prepared by treatment with an excess of NaH in boiling THF, followed by filtration under N_2. This solution was then added to $(NPCl_2)_3$ in THF (method A). [b] The THF was removed at reduced pressure, the residue was dissolved in benzene, the mixture was filtered, the benzene was removed, and the oil was filtered from unreacted $(NPCl_2)_3$ and VI. [c] Oil at 25 °C. [d] Yield based on ^{31}P NMR analysis of the product mixture. [e] The sodium salt of the steroid was prepared by treatment with excess NaH in THF at 25 °C for 8 h, followed by 2 h at reflux and filtration under N_2. [f] Added by means of a dry ice condenser to the reaction mixture at 0 °C. The reaction was allowed to proceed for 2 h as the mixture warmed to 25 °C and for a further 36-48 h at 25 °C. [g] Isolation by filtration, removal of solvent, and precipitation of the product by addition of acetone. [h] Dissolution in benzene, filtration, concentration, and precipitation with acetone; precipitation from THF by acetone. [i] White crystalline solid. [j] Addition by method A. [k] Filtered and concentrated by use of a rotary evaporator. [l] Addition by method B. [m] XX and XXI were formed in a 2:3 ratio and XXII and XXIII in a 1:1 ratio. [n] Isomers XXII and XXIII were present in a 1:1 ratio. [o] XXVI and XXVII in a 3:2 ratio were cocrystallized from methylene chloride/pentane. [p] XXVI and XXV were present in a 1:4 ratio, by ^{31}P NMR analysis. [q] The THF was removed at reduced pressure, and the residue was washed with water, hot methanol, and hot acetone, was precipitated twice from methylene chloride into pentane, and was recrystallized from mixtures of these two solvents. [r] Impurities were removed by successive extractions of the solid or oil with ethanol and by solution in benzene, followed by precipitation with petroleum ether. [s] The sodium salt of VIII was prepared by treatment with excess NaH for 2 h at 25 °C, after which the filtered solution was added to the solution of $(NPCl_2)_3$. [t] The product was isolated by successive dissolutions of the concentrated reaction mixture in methylene chloride, precipitation with petroleum ether, column chromatography using neutral alumina with chloroform and then ethanol, precipitation from hot 95% ethanol into petroleum ether, and recrystallization from methylene chloride/petroleum ether. [u] Ultraviolet peaks (in diethyl ether) at 277.2 and 269.6 nm; IR peak at 1200 cm^{-1} (N_3P_3 ring). [v] Ultraviolet peaks (in THF) at 275.5, 267.8, and 209 nm; IR peak at 1190 cm^{-1} (N_3P_3 ring). [w] IR peak at 1195 cm^{-1}.

and water, a white crystalline material, with a melting point between 135 and 136 °C,[32] was obtained in 60% yield. The mass spectrum showed a parent peak at 255 amu (calcd for VI, 255 amu). An infrared spectrum showed no carbonyl absorbance. Ultraviolet absorbance maxima were found at 291 and 282 nm (in diethyl ether).

General Reaction Conditions. The overall synthetic procedure involved an initial interaction of the steroid in THF with sodium hydride either at 25 °C or at reflux temperature. The unreacted sodium hydride was removed by filtration and washed with THF, and the combined filtrates were allowed to react with a solution of $(NPCl_2)_3$ in THF. After several hours of reaction at reflux temperature or at 25 °C, one of two alternative procedures was then followed. Either the reaction mixture was concentrated, filtered, and studied spectroscopically or by mass spectrometry, or the reaction solution was cooled to 0 °C as methylamine was added via a dry ice condenser. In the latter case, the mixture was stirred and cooled for several hours and was then allowed to warm to room temperature over a period of 36–48 h. The mixture was then filtered and concentrated, and the products (V) were purified by precipitation and crystallization. Characterization was effected by ultraviolet or NMR spectroscopy, by mass spectrometry, and by microanalysis. Details for specific reactions are listed in Table I, and the ^{31}P NMR data are tabulated in Table II. Special procedures and comments are contained in the following sections.

Experimental Conditions for Steroids VI–XV. Methods A and B. Because of the low solubilities of the steroid salts derived from VI–XV (~0.007 M in THF), two general methods were used to prepare compounds with the general structure IV. Method A involved the addition of a saturated solution of the steroid salt derived from VI–XV (prepared from the steroid and sodium hydride in THF) to an excess of $(NPCl_2)_3$ (III). In Method B, the steroidal alcohol, $(NPCl_2)_3$, and excess sodium hydride were mixed in THF and allowed to react. These conditions provided an equilibrium concentration of the sodium salt of the steroid in the reaction mixture. Sodium hydride did not react with $(NPCl_2)_3$ even when boiled at reflux in THF or 1,4-dioxane. Compounds with the structure IV were soluble in THF or dioxane.

Reaction of $(NPCl_2)_3$ (III) with the Sodium Salt of Estrone (VII) (1:1 Molar Ratio). Method A. Following the dropwise addition of the sodium salt of VII to $(NPCl_2)_3$, the mixture was concentrated, and a ^{31}P NMR spectrum of this mixture was interpreted as an AB_2 spin system derived from IV (where the residue derived from estrone was linked through the C-3 aryl oxygen atom) and an A_3 spin system, assigned to unreacted $(NPCl_2)_3$.

Reactions of $(NPCl_2)_3$ (III) with the Sodium Salt of Estrone (12 Equiv). Method B. After filtration and concentration of the reaction mixture, compounds XIX–XXIII were identified by ^{31}P NMR comparisons with the products derived from the reactions of $(NPCl_2)_3$ with sodium trifluoroethoxide[28] or sodium phenoxide. The isomeric pairs XX and XXI were formed in a 40:60 ratio (see Table II). However, the stereochemistry of the predominant isomer was not established. The isomers could be identified because they had very similar (but not identical) chemical shifts and coupling constants. Small quantities of isomers XXII and XXIII were also formed in a 1:1 ratio. The ^{31}P NMR data are listed in Table II. In other related reactions (see Tables I and II), species XXVI and XXVII were identified

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Table II. ^{31}P NMR Chemical Shifts and Coupling Constants[a,b]

compd	spin system	A	B	A − B	J_{AB}	$J_{AB/\Delta\nu}$
Control Compounds						
$(NPCl_2)_3$ (III)	A_3	19.8				
$N_3P_3Cl_5(OCH_2CF_3)$[24]	AB_2	14.1	20.4	6.24	66.8	0.26
$N_3P_3Cl_5(OC_6H_5)$[c]	AB_2	9.9	19.9	9.95	62.8	0.16
$N_3P_3Cl_5(OC_6H_4Br\text{-}p)$[d]	AB_2	12.1	22.1	10.0	61.6	0.15
$N_3P_3Cl_5$(OH or Na)[c]	AB_2	0.4	19.1	18.7	64.4	0.085
	AB_2	1.3	20.5	19.2	65.2	0.084
$N_3P_3Cl_4(OCH_2CF_3)_2$	AB_2 (trans nongeminal)[26]	25.3	19.4	5.9	69.3	0.29
$N_3P_3Cl_4$(OH or ONa)$_2$	AB_2 (geminal)[c]	−4.2	16.9	21.1	49.0	0.057
$N_3P_3Cl_3(OCH_2CF_3)_3$	AB_2 (trans nongeminal)[26]	17.8	17.9			
$N_3P_3Cl_2(OCH_2CF_3)_4$	AB_2 (nongeminal)[26]	11.6	24.3	12.7	85.9	0.17
$N_3P_3Cl(OCH_2CF_3)_5$[c]	AB_2	26.0	14.0	12.0	88.3	0.18
$N_3P_3Cl(OC_6H_5)_5$	AB_2	22.1	6.9	15.2	83.4	0.14
$[NP(OCH_2CF_3)_2]_3$[c]	A_3	18.0				
$[NP(OC_6H_5)_2]_3$[c]	A_3	8.7				
$[NP(NHCH_3)_2]_3$	A_3	23.0				
$N_3P_3(NHCH_3)_5(OCH_2CF_3)$[c]	AB_2	23.0	21.2	2.1	55.1	0.65
$N_3P_3(NHCH_3)_5$(ONa or OH)[g]	AB_2	7.8	19.3	11.5	28.8	0.062
$N_3P_3(NHCH_3)_4$(ONa or OH)$_2$[g]	AB_2	1.1	17.5	15.9	35.2	0.055
	$N_3P_3Cl_5(OR)$					
HOR = VI[e]	AB_2	11.7	21.7	10.0	60.3	0.15
VII[c]	AB_2	11.9	21.9	10.0	61.2	0.15
VIII[c]	AB_2	12.0	22.0	10.0	61.2	0.15
IX[c]	AB_2	11.8	22.0	10.2	62.0	0.15
X[c]	AB_2	13.1	22.3	9.2	63.6	0.17
XI[c]	AB_2	12.4	22.7	10.3	60.8	0.15
XIV[c]	AB_2	14.2	21.5	7.3	62.8	0.21
	$N_3P_3Cl_4(OR)_2$					
HOR = VII[c] (nongeminal, two isomers)						
isomer a	AB_2	24.8	15.1	9.7	68.0	0.17
isomer b	AB_2	24.3	14.6	9.7	66.4	0.17
	$N_3P_3Cl_3(OR)_3$					
HOR = VII[c] (nongeminal, two isomers)						
	singlet	17.9				
	singlet	17.5				
	$N_3P_3Cl_2(OR)_4$					
HOR = VII[c] (nongeminal, two isomers)						
	AB_2	4.4	19.6	15.2	79.8	0.13
	AB_2	4.7	19.7	15.0	81.4	0.13
	$N_3P_3Cl(OR)_5$					
HOR = VII[c]	AB_2	21.1	6.15	14.95	85.1	0.14
	$N_3P_3(OR)_6$					
HOR = VII[c]	A_3	8.4				
	$N_3P_3(NHCH_3)_5(OR)$[f]					
HOR = VI[c]	AB_2	17.7	18.8	1.10	55.5	1.20
VI[g]	AB_2	19.0	19.9	0.90	52.2	1.40
VI[h]	AB_2	21.6	22.8	1.20	50.2	1.00
VII[c]	AB_2	19.5	20.5	1.00	55.1	1.40
VII[g]	AB_2	19.0	19.9	0.90	52.2	1.40
VIII[c]	AB_2	20.2	21.2	1.00	55.1	1.40
IX[c]	AB_2	21.2	22.1	0.90	55.1	1.50
X[g]	AB_2	17.7	17.7			
XIV[c]	AB_2	18.4	18.4			
XIV[g]	AB_2	18.7	18.7			
XIV[h]	AB_2	21.4	21.4			
XV[c]	AB_2	17.5	17.5			

[a] All the spectra were obtained in a proton-decoupled mode with the use of a D_2O-capillary lock. Chemical shifts were relative to an external 85% phosphoric acid reference. Positive shifts are downfield. [b] Assignments for chemical shifts (ppm) and coupling constants (Hz) were determined for the AB_2 spin systems by comparison with published computer simulations and with the use of the following equations: $\Delta\nu = \nu_A - \nu_B$ (ppm), where ν_A = line 3 and ν_B = (line 5 + line 7)/(2; $J_{AB} = {}^1/_3[(\nu_8 - \nu_6) + (\nu_4 - \nu_1)]$.[27] [c] In tetrahydrofuran. [d] The spectrum was obtained for the liquid. [e] The sample was dissolved in benzene-d_6, and no D_2O-capillary lock was employed. [f] These spectra were not sufficiently well resolved to allow exact assignments to be more directly. However, computer simulations and an iterative calculation process based on observed frequencies allowed the correct assignments for the coupling constants and the chemical shifts to be determined. The assignments were made with the use of the computer program NMR,LMA written by W. F. Slivinski, D. L. Doerfler, and K. J. Johnson which was used to simulate the AB_2 NMR spectra. [g] In ethanol. [h] In benzene.

by a comparison with the ^{31}P NMR spectra of authentic samples of $N_3P_3Cl(OC_6H_5)_5$ and $[NP(OC_6H_5)_2]_3$.

The identity of XXVII was verified by the similarity of its ^{31}P NMR spectrum to that of $[NP(OC_6H_5)_2]_3$ (Table II). The chlorine content of this material was shown by analysis to be less than 0.09%.

Reaction of $(NPCl_2)_3$ with the Sodium Salt of Estrone (VII) and Methylamine. Method A. Estrone (3 g, 0.011 mol) was dissolved in anhydrous THF (100 mL), and the solution was stirred at 27 °C for 2 h with excess sodium hydride. The suspension was filtered under nitrogen, and the filtrate was added to a stirred solution of $(NPCl_2)_3$ (5.13 g, 0.0148 mol) in THF (25 mL). Twelve hours later, methylamine (300 mL) was added with the use of a dry ice condenser, and the mixture was stirred for 48 h. The solvent and unreacted methylamine were allowed to evaporate, and the residue was dissolved in benzene. After filtration and evaporation of the benzene, a solid residue was obtained which was identified as the species of structure V, where OR was the methylimino derivative of estrone bonded through the 3-oxy group. (The methylimino group had a strong infrared absorbance at 1670 cm^{-1}.) Further characterization of the solid was performed after conversion of the methylimino derivative to the ketone. The solid was stirred in boiling water, and the mixture was filtered. The water was evaporated from the filtrate at 100 °C (with the use of a rotary evaporator) to yield a white residue which was soluble in ethanol and THF. Petroleum ether was used to precipitate the product from a concentrated solution in THF. The precipitate was isolated and was redissolved in hot water, filtered, and the water was evaporated from the filtrate. A quantitative conversion of the methylimino derivative (water insoluble) to the ketone (water soluble) was possible, as demonstrated by the use of infrared spectroscopy. The carbonyl group had a strong absorbance at 1740 cm^{-1}. A final reprecipitation of the water-soluble residue from THF by the addition of petroleum ether gave a white material that melted at 155 °C and was analytically pure. The overall yield was 20%. A crystal–crystal transition was detected at 137 °C. A mass spectrum showed a parent ion at m/e 554 (theory for V where HOR is VII m/e 554). An infrared P=N stretching peak was detected at 1190 cm^{-1}. Anal. Calcd for $C_{23}H_{41}N_8O_2P_3$: C, 49.82; H, 7.40; N, 20.22; P, 16.79; Cl, 0; O, 5.78. Found: C, 49.99; H, 7.39; N, 19.94; P, 16.69; Cl, 0; O (by difference), 5.99. A ^{31}P NMR spectrum of the product was interpreted as an AB_2 spin pattern (see Table II).

Reaction of $(NPCl_2)_3$ with the Sodium Salt of Estradiol 3-Methyl Ether (XIV) and Methylamine. The reaction conditions are listed in Table I. Anal. Calcd for $C_{24}H_{45}N_8O_2P_3$ (V): C, 50.53; H, 7.89; N, 19.65; P, 16.32; Cl, 0; O, 5.61. Found: C, 50.53; H, 8.03; N, 19.52; P, 16.30; Cl, 0; O (by difference), 5.63. The ^{31}P NMR spectrum of the product was interpreted as an AB_2-type pattern (Table II).

The steroid 1,4-dihydroestradiol 3-methyl ether (XV) reacted with sodium hydride, $(NPCl_2)_3$, and methylamine to give V under similar reaction conditions. The product was characterized with the use of ^{31}P NMR methods (see Table II).

Reaction of $(NPCl_2)_3$ with the Sodium Salt of β-Estradiol (VIII) and Methylamine. The reaction conditions are listed in Table I. Anal. Calcd for $C_{23}H_{43}N_8O_2P_3$ (V): C, 49.64; H, 7.73; N, 20.14; P, 16.73; O, 5.75. Found: C, 48.44; H, 7.75; N, 18.94; P, 15.87. A ^{31}P NMR spectrum of the product was compatible with an AB_2 spin pattern. The chemical shifts and coupling constant are listed in Table II.

Reactions of $(NPCl_2)_3$ with the Sodium Salt of α-Ethynylestradiol (IX) and Methylamine. See Table I for the reaction conditions. Anal. Calcd for $C_{25}H_{43}N_8O_2P_3$ (V): C, 51.72; H, 7.41; N, 19.31; P, 16.03; O, 5.52. Found: C, 50.55; H, 8.14; N, 18.01; P, 15.69. A ^{31}P NMR spectrum of the product was interpreted as an AB_2 spin system (see Table II).

Reaction of $(NPCl_2)_3$ with Cholesterol (X). Cholesterol (2.111 g, 0.00546 mol) was dissolved in THF (100 mL), and an excess of sodium hydride was added. Under nitrogen, the suspension was boiled at reflux for 5 h and was then filtered. The filtrate was added dropwise to a solution of $(NPCl_2)_3$ (1.9 g, 0.005 46 mol) dissolved in THF (100 mL), and the mixture was stirred at 27 °C for 12 h. A concentrate of the pale yellow turbid reaction mixture gave a ^{31}P NMR spectrum which suggested that 23% of the $(NPCl_2)_3$ had reacted, but the spectrum was inconsistent with the structure of a steroid–phosphazene ester such as IV (where HOR = cholesterol). It was interpreted as an AB_2 spin pattern compatible with the possible structure $N_3P_3Cl_4(OH)_2$ (see Table II). Unreacted cholesterol (1.5 g, mp 143 °C) was recovered following its precipitation by the addition of pentane to the concentrate. A mass spectrum of a fraction of the heptane-soluble residue, isolated by chromatography with silica gel and hexane, showed a parent ion at m/e 369 which was the ion from 3,5-cholestadiene (theory m/e 368). This fraction had a melting point range from 78 to 80 °C and an ultraviolet absorbance maximum at 234 nm (in hexane). (3,5-Cholestadiene melts at 80 °C and has an absorbance maximum at 234 nm in cyclohexane.) Less than a 5% yield of the anticipated compound IV (where HOR = cholesterol) was obtained, as indicated by ^{31}P NMR spectroscopy (see Table II). In addition, $(NPCl_2)_3$ was identified from the mass spectral Cl_6-isotope pattern found at 345 amu.

Reaction of $(NPCl_2)_3$ with Cholesterol (X). Method B. Cholesterol (5 g, 0.013 mol), excess sodium hydride (5 g, 0.217 mol), and $(NPCl_2)_3$ (3 g, 0.0086 mol) were combined, and THF (300 mL) was added. The reaction mixture was stirred for 1 week and was then filtered under nitrogen. The filtrate was concentrated with the use of a rotary evaporator and was analyzed by ^{31}P NMR methods. The compounds $N_3P_3Cl_5(ONa)$, $N_3P_3Cl_5(OR)$, and possibly $N_3P_3Cl_4(ONa)_2$ (geminal) were detected (see Table II). The latter two compounds were formed in a 1:1 ratio. After the solvent had been removed, cholesteryl chloride was isolated by chromatography (silica/hexane) and by fractional recrystallization (pentane). This compound was identified by melting point [90–97 °C (lit. 97 °C)] and by mass spectrometry. A parent ion was found in the mass spectrum at m/e 405 (calcd for $C_{27}H_{45}Cl$ m/e 405). Unreacted cholesterol (mp 143–146 °C) was also recovered. Small quantities of cholestadiene were also isolated from the reaction mixture by chromatography (silica/hexane). This compound was identified by melting point (78–80 °C) and by mass spectrometry. A parent ion at m/e 368 was found in the mass spectrum (calcd for cholestadiene, $C_{27}H_{44}$, m/e 368). The compound $N_3P_3Cl_5(OR)$ (HOR = X) was stable to boiling THF for 10 min. The species believed to be $N_3P_3Cl_4(ONa)_2$ was unstable to small amounts of added sodium hydroxide or hydrochloric acid. The species $N_3P_3Cl_5(OR)$ was stable under the same conditions.[24]

The reaction mixture was characterized further after treatment with methylamine. A portion of the reaction mixture was dissolved in THF (100 mL) and was cooled to 0 °C with an ice bath. Methylamine (200 mL) was added with the use of a dry ice condenser. After 47 h, the solvent was removed on a rotary evaporator, and the residue was dissolved in ethanol (95%). A ^{31}P NMR spectrum of the mixture was used to identify $[NP(NHCH_3)_2]_3$, possibly $N_3P_3(NHCH_3)_4(ONa)_2$, and $N_3P_3(NHCH_3)_5(OR)$ (V), where HOR = X. The ^{31}P NMR data are listed in Table II. The mixture was separated by chromatography (neutral alumina/ethanol). One fraction contained a white solid (mp 157 °C, 20 mg). A ^{31}P NMR spectrum was consistent with structure V, where HOR = X (Table II). A mass spectrum of the material had a parent peak at m/e 368, attributed to the ion from cholestadiene (mol wt 368). Thus, the formation of IV and V, where HOR = X, was complicated by side reactions and by difficulties encountered during the purification procedures. Compounds XI–XIII behaved similarly.

Comparison of the Reactions of Cholesterol (X), Dihydrocholesterol (XI), Estradiol 3-Methyl Ether (XIV), Estrone 3-Methyl Ether (XVI), and Mestranol (XVII) with $(NPCl_2)_3$. Method B. A solution of the steroid X, XI, XIV, or XV (1.00 g) and $(NPCl_2)_3$ (1.00 g, 0.002 87 mol) dissolved in THF (300 mL) was stirred with a suspension of excess sodium hydride at 27 °C for 24 h. The reaction mixture was filtered, and the filtrate was examined by ^{31}P NMR methods. None of the steroids reacted with III during this time. After 2 weeks at 27 °C, the reaction mixtures were filtered, and the filtrates were studied by ^{31}P NMR spectrometry. Mestranol had not reacted appreciably under these conditions. Cholesterol (X), dihydrocholesterol (XI), and estradiol 3-methyl ether (XIV) had reacted with approximately 25% of the $(NPCl_2)_3$ to form IV in <5%, 5%, and 20% yields, respectively. Cholesterol (X) and dihydrocholesterol (XI) reacted with $(NPCl_2)_3$ to form hydroxyphosphazenes and related products. Estrone 3-methyl ether (XVI) reacted with <5% of the $(NPCl_2)_3$ to form hydroxyphosphazenes. Thus, the reaction

of sodium hydride with steroids X, XI, XIV, and XVI was a slow process at 25 °C, as was the reaction of the steroidal alcohols with $(NPCl_2)_3$. Cholesterol (or IV, where HOR = X) dehydrated faster than dihydrocholesterol (or IV, where HOR = XI), which in turn dehydrated faster than estradiol 3-methyl ether (or IV, where HOR = XIV). Estrone 3-methyl ether (XVI) and mestranol were slow to react.

$(NPCl_2)_3$ as a Dehydration Agent for *tert*-Butyl Alcohol. *tert*-Butyl alcohol (40 mL) was mixed with sodium (0.3 g, 0.0130 mol) and was boiled at reflux for 10.5 h. THF (50 mL) was added to the alkoxide, and to this solution was added rapidly a solution of $(NPCl_2)_3$ (4 g, 0.0115 mol) dissolved in THF (40 mL). The system was heated to reflux, the solution became cloudy, and a gas was collected from the reaction mixture in a liquid nitrogen trap. The gas was identified as isobutylene by infrared spectroscopy.

Sodium *tert*-butoxide (0.241 g, 0.002 51 mol) was dissolved in THF (50 mL) and was added dropwise to a stirred solution of $(NPCl_2)_3$ (1.0 g, 0.002 87 mol) in THF (50 mL). A ^{31}P NMR spectrum of a concentrate of the reaction mixture showed only unreacted $(NPCl_2)_3$. Thus, it was concluded that *tert*-butoxide reacts only slowly with $(NPCl_2)_3$ at 25 °C.

Interaction of Pregnenolone (XII) or XIII with $(NPCl_2)_3$. Serious complications were encountered when attempts were made to bring about a reaction between $(NPCl_2)_3$ and the sodium salts of XII or XIII. These problems were attributed (1) to the difficulty of formation of the monosodium salts of XII or XIII, (2) to the possible dehydration of XII (as discussed earlier for cholesterol), (3) to the reactivity of the acetyl group at position 17 in XII and XIII, and (4) to the low solubility of the sodium salt derived from XIII.

For example, pregnenolone (5 g, 15.8 mmol) was allowed to react with sodium hydride (5 g, 0.208 mol) in THF at reflux temperature for 4 h. The mixture was filtered at 25 °C. Treatment with methyl iodide yielded a product that showed clear infrared spectral evidence for methoxy groups (at position 3) and for the methyl ether derived by keto–enol tautomerism from a $C(OH)CH_3$ unit at position 17. Thus, both the 3- and 17-positions of XII appeared to possess sites of reactivity in the presence of sodium hydride.

A similar mixture derived from XII and sodium hydride was added dropwise to a rapidly stirred solution of $(NPCl_2)_3$ (6.00 g, 17.2 mmol) at 0 °C in THF (150 mL) during 1 h. The mixture was then stirred for 10 h at 25 °C. An excess of liquid methylamine was added by means of a dry ice condenser, and the mixture was stirred at 0 °C for 1 h and then at 25 °C for 24 h. Removal of the THF was followed by solution in benzene, filtration, evaporation of the solvent, extraction with ethanol, and extraction with petroleum ether. The extract gave a 2% yield of a product (mp 82–84 °C) that showed infrared and ^{31}P NMR spectra which are compatible with a species of structure V but which could not be separated sufficiently well from the $[NP(NHCH_3)_2]_3$ impurity to yield a satisfactory microanalysis. Evidence was also obtained that the methylimino derivative was formed initially at position 20.

The sodium salt of XIII and of the 20-ethylene ketal protected derivative proved to be quite insoluble in THF at 25 °C or at lower temperatures. Although reactions were attempted between these salts and $(NPCl_2)_3$, the product yields were extremely low. Similar problems were encountered when attempts were made to induce the reaction between the sodium salt of cyclohexanol and $(NPCl_2)_3$.

Structural Proof. General Composition. The steroid-substituted cyclic species $N_3P_3Cl_5(OR)$ (IV) and $N_3P_3(NHCH_3)_5(OR)$ (where HOR = VI–IX or XIV) were characterized by ^{31}P NMR spectroscopy, by mass spectrometry, and by infrared and ultraviolet spectroscopy. Microanalytical agreement was obtained for the composition of compounds of structure V derived from VII–IX and XIV. The compounds V (where HOR = X and XV) were characterized only by ^{31}P NMR and infrared techniques.

The mass spectrometric data were consistent with the expected elemental compositions of structures IV and V in which the OR groups were derived from VI–IX and XIV. Strong parent peaks were found in each case. The typical high mass fragmentation patterns showed the successive loss of from one to three chloro- or methylamino groups to form fragments of the type $[N_3P_3(OR)Cl_{5-x}]^+\cdot$ or $[N_3P_3(OR)(NHCH_3)_{5-x}]^+\cdot$ (where x = 1–3). For the species of type V, the fragmentation patterns also showed a successive loss of methyl groups. Mass spectrometry provided no firm evidence for the presence of compounds of types IV and V where the OR unit was derived from X–XIII, XVI, or XVII.

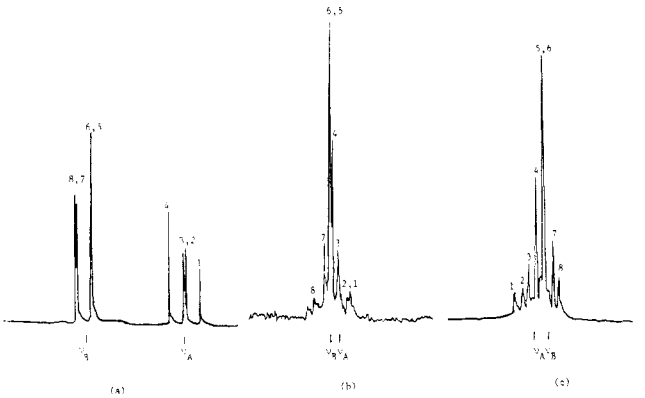

Figure 1. ^{31}P NMR spectra are shown for compounds (a) IV (OR = OC_6H_5), (b) V (OR = the residue derived from VI), and (c) V (OR = OCH_2CF_3). They represent typical AB_2-spin patterns observed for compounds with the structure IV or V (see Table IV-1) of ref 27. The limiting AB_2 spectrum ($J/\Delta\nu = \infty$) is a singlet, and this was observed for the compound V (OR = the residues derived from X, XIV, or XV).

The survival of the phosphazene ring during the substitution reaction was confirmed by the infrared P–N stretching frequencies in the 1175–1216-cm^{-1} region for IV and V. No significant differences could be discerned between the P–N absorption frequencies of the various species of general formula V (1195 cm^{-1}). Infrared spectroscopy was used to identify the 17-methylimino residue of the side group derived from estrone (VII) in species V and to follow the subsequent hydrolysis of this unit back to the carbonyl function.

The cyclophosphazene ring has no significant ultraviolet absorption at wavelengths longer than 220 nm. Hence, the substitution of chlorine by VI or VII could be monitored by ultraviolet spectroscopy. The linkage of residues from steroids VI and VII to the phosphazene ring of IV or V resulted in a hypsochromic shift from 290 nm in the free steroid to 275.5 nm in THF. By contrast, no ultraviolet spectral shift took place when XIV was attached to the phosphazene ring.

Structural Proof. NMR Spectra. The ^{31}P NMR spectra of IV (HOR = VI–IX, X, XI, and XIV; Table II) were interpreted as AB_2 spin systems, comparable to those of $N_3P_3Cl_5(OCH_2CF_3)$[26] or $N_3P_3Cl_5(OC_6H_5)$. Similarly, the spectra of species V (HOR = VI–IX, X–XI, XIV, and XV) were comparable to the AB_2 spin pattern of $N_3P_3(NHCH_3)_5(OCH_2CF_3)$. Like other AB_2 spin systems, the chemical shifts and coupling constants for these compounds were both solvent and concentration dependent. Specific values are given in Table II, and the general patterns of these spectra are shown in Figure 1.[27] In general, the ^{31}P chemical shifts for $N_3P_3Cl_5X$ (values in parts per million in parentheses) were in the order: X = Cl (20.0) > residue from XIV (14.2) ≃ OCH_2CF_3 (14.1) > residue from X (13.1) ≃ residue from XI (12.4) > residues from VI–IX (11.7–12.0) > OC_6H_5 (9.9). This parallels the expected order of increasing electron supply from the side group to phosphorus in the manner seen for other cyclophosphazene derivatives.[28] The replacement of chlorine in IV by methylamino to give V markedly reduced the chemical shift difference between the A and B_2 phosphorus atoms and brought about an overlap of the two.

^{13}C NMR spectra were used to confirm the retention of the steroidal framework following the linkage and co-substitution reactions. Although the ^{13}C NMR spectra were not sufficiently resolved to permit the identification of every carbon nucleus[29] in the steroid structure for $N_3P_3(OR)Cl_5$ (IV) or $N_3P_3(OR)(NMeH)_5$ (V) (where HOR = VI, VII, and XIV), no change in the absorbances of the aromatic nuclei for steroid XIV occurred as a result of the interaction with sodium hydride or with $(NPCl_2)_3$. This indicated that no major side reactions had occurred that involved the ether function at the 3-position of XIV. For steroid XIV, only a slight change in the relative intensity and no change in the chemical shift at 82 ppm was observed for the ^{13}C nucleus

at the 17-position after attachment to the phosphazene ring. By contrast, changes in the chemical shifts for the resonances of aromatic ^{13}C nuclei for steroid VI were detected following reaction of the sodium salt of VI with $(NPCl_2)_3$. ^{13}C NMR spectroscopy (together with mass spectrometry) was also used to confirm the identity of the unreacted steroids VI, VII, and XIV isolated from the reaction products. This confirmed the absence of side reactions. No mass spectral or ^{13}C NMR spectral changes occurred after XVII had been treated with liquid methylamine (for 72 h).

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Registry No. III, 940-71-6; IV (HOR = VI), 74026-89-4; IV (HOR = VII), 75267-32-2; IV (HOR = VIII), 75267-33-3; IV (HOR = XIV), 75267-34-4; V (HOR = VI), 74026-90-7; V (HOR = VII), 74026-91-8; V (HOR = VIII), 74026-95-2; V (HOR = IX), 75267-35-5; V (HOR = X), 74026-93-0; V (HOR = XIV), 75283-90-8; V (HOR = XV), 75267-37-7; VI, 53-63-4; VI sodium salt, 74026-88-3; VII, 53-16-7; VII sodium salt, 74040-94-1; VIII, 50-28-2; VIII sodium salt, 17181-19-0; IX, 57-63-6; IX sodium salt, 75267-38-8; X, 57-88-5; XI, 17608-41-2; XII sodium salt, 75267-39-9; XIII sodium salt, 75267-40-2; XIV, 1035-77-4; XIV sodium salt, 74026-92-9; XVI, 1624-62-0; XVII, 72-33-3; XIX (HOR = IX), 75267-41-3; XIX (HOR = X), 75267-42-4; XIX (HOR = XI), 75267-43-5; XX (HOR = VII), 75267-44-6; XXI (HOR = VII), 75330-94-8; XXII (HOR = VII), 75299-11-5; XXIII (HOR = VII), 75363-53-0; XXIV (HOR = VII), 75283-91-9; XXV (HOR = VII), 75331-70-3; XXVI (HOR = VII), 75267-45-7; XXVII (HOR = VII), 75267-46-8; $N_3P_3Cl_5(OCH_2CF_3)$, 13053-90-2; $N_3P_3Cl_5(OC_6H_5)$, 3028-10-2; $N_3P_3Cl_5(OC_6H_4Br\text{-}p)$, 3887-16-9; $N_3P_3Cl_5(OH)$, 75267-47-9; $N_3P_3Cl_5(ONa)$, 75283-92-0; $N_3P_3Cl_4(OCH_2CF_3)_2$, 75267-48-0; $N_3P_3Cl_4(OH)_2$, 75267-49-1; $N_3P_3Cl_4(ONa)_2$, 75267-50-4; $N_3P_3Cl_3(OCH_2CF_3)_3$, 75330-95-9; $N_3P_3Cl_2(OCH_2CF_3)_4$, 75267-51-5; $N_3P_3Cl(OCH_2CF_3)_5$, 55975-53-6; $N_3P_3Cl(OC_6H_5)_5$, 5032-39-3; $[NP(OCH_2CF_3)_2]_3$, 1065-05-0; $[NP(OC_6H_5)_2]_3$, 1184-10-7; $[NP(NHCH_3)_2]_3$, 1635-63-8; $N_3P_3(NHCH_3)_5(OCH_2CF_3)$, 75283-93-1; $N_3P_3(NHCH_3)_5(OH)$, 75267-52-6; $N_3P_3(NHCH_3)_5(ONa)$, 75267-53-7; $N_3P_3(NHCH_3)_4(OH)$, 75267-54-8; $N_3P_3(NHCH_3)_4(ONa)_2$, 75267-55-9.

Ichthyotoxic and Cytotoxic Metabolites of the Tropical Brown Alga *Stypopodium zonale* (Lamouroux) Papenfuss[1]

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Seven new natural products, stypoldione, stypotriol, stypodiol, epistypodiol, epitaondiol, 2-(geranylgeranyl)-5-methyl-1,4-benzohydroquinone, and 2-(geranylgeranyl)-5-methyl-1,4-benzoquinone, and two previously reported metabolites, taondiol and atomaric acid, have been isolated from the Caribbean brown alga *Stypopodium zonale*. Taondiol was obtained as the enantiomer of that previously reported. The structures of the new compounds were based upon the previously described X-ray structure of stypoldione and upon detailed ^{13}C and ^1H NMR analyses. Nearly all the compounds isolated showed toxic or strong narcotic effects upon the reef-dwelling fish, *Eupomacentrus leucostictus*, and stypoldione was found to be an extremely potent inhibitor of synchronous cell division in a fertilized sea urchin egg assay.

In the course of our investigations of the chemical defense strategies of tropical marine algae, we are continuing to explore the natural products chemistry and subsequent chemical ecology of brown seaweeds of the family Dictyotaceae (Dictyotales). One such example in this family is the brown alga *Stypopodium zonale* (Lamouroux) Papenfuss, which is found to grow luxuriantly in the Western Caribbean Sea where herbivorous invertebrates and fishes are particularly abundant.[2] In an earlier communication, we reported the structures of two ichthyotoxic metabolites, stypoldione and stypotriol, isolated from *S. zonale*.[3] In this complete report of our investigations of this alga, we describe the structures of several new biologically active compounds and amplify upon our initial findings.

Stypopodium zonale was initially chosen for study based on observations that when the freshly collected plant was placed in a well-aerated aquarium, the alga was found to excrete rust-colored substances which proved to be toxic to the Caribbean herbivorous fish *Eupomacentrus leucostictus*. During the course of 1 week, with daily changes of the aquarium water, the plants continued to excrete ichthyotoxic substances with no visible degeneration of the plants themselves. Diethyl ether extraction of the water yielded a complex mixture of UV-absorbing compounds, the major component of which was the bright red *o*-quinone, stypoldione (1). Stypoldione in seawater, at 1.0 μg/mL, induced the same toxic symptoms as the algae-treated water.

The chloroform–methanol extract of the fresh alga proved even more toxic than pure 1, suggesting that a more potent intracellular toxin was present. Indeed, when algal extracts were rapidly chromatographed in the field, a white solid, stypotriol (2), was obtained, which rapidly began turning pink and then red. The hydroquinone 2 was substantially more toxic than 1 (LD $\simeq$ 0.2 μg/mL), but as indicated by the color change, it is rapidly air oxidized to stypoldione such that precise concentrations in seawater cannot be produced.[4] Hence, the major toxic metabolite of *S. zonale* is the *o*-hydroquinone 2, which when excreted, is rapidly air oxidized to the *o*-quinone 1. The structures of 1 and 2 were communicated earlier[3] based upon a conclusive X-ray analysis of 1. Complete analysis of the extract has now resulted in the isolation of compounds 2 and 4–10 (Chart I), and several of these compounds contribute to the observed overall ichthyotoxicity of *S. zonale*. Contrary to our earlier report, the alga does contain taondiol (9),[5] as well as atomaric acid (10);[6] however, the

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(4) The details of this fish toxicity bioassay were recently summarized, see: Sun, H. H.; Fenical, W. *Tetrahedron Lett.* **1979**, 685.

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