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SOME STEREOCHEMICAL FACTORS IN THE FORMATION OF REARRANGEMENT IONS IN THE MASS SPECTRA OF TRIMETHYLSILYL DERIVATIVES OF STEROIDAL PHOSPHATES

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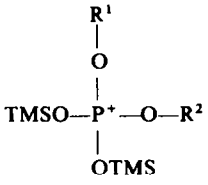
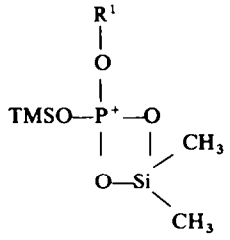
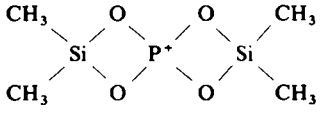
Abstract—The mass spectra of trimethylsilyl derivatives of organic phosphates exhibit characteristic rearrangement ions involving both trimethylsilyl and hydrogen migrations to the phosphate moiety. It is shown that in molecules of relatively rigid structure, such as steroids, the processes leading to the formation of the rearrangement ions are dependent on the positioning of the functional groups.

THE MASS SPECTROMETRY of trimethylsilyl (TMS) derivatives of sugar phosphates[1–4] and nucleotides[5–8] has been a subject of recent interest. A characteristic feature of the mass spectra of these derivatives is the presence in high abundance of several phosphorus-containing rearrangement ions, notably those of mass 387, 315, 299, 243, 227 and 211 (**a–f**). Most of these ions can be formed by a variety of mechanisms





$R^1 = R^2 = TMS$ (**a**, *m/e* 387) $R^1 = TMS$ (**d**, *m/e* 299)
$R^1 = H$, $R^2 = TMS$ (**b**, *m/e* 315) $R^1 = H$ (**e**, *m/e* 227)
$R^1 = R^2 = H$ (**c**, *m/e* 243)

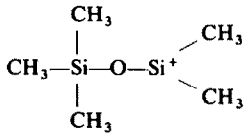


f, *m/e* 211

from several precursor ions,[2,8] and their formation usually involves both intramolecular and intermolecular[9,10] trimethylsilyl and/or hydrogen migrations.

Recent studies have indicated that the abundance of an ion (*m/e* 147, **g**) whose formation involves transfer of a trimethylsilyl group is stereochemically dependent.[11,12] In view of these results it is probable that the relative abundance of

the phosphate rearrangement ions (**a–e**) should also be dependent on the stereochemical disposition of the TMS and trimethylsilylphosphate groups. Since the rigid structure of the steroid molecule offers an ideal system for investigating this possibility, the mass spectra of the trimethylsilyl derivatives of several steroidal phosphates were studied.



g, *m/e* 147

RESULTS AND DISCUSSION

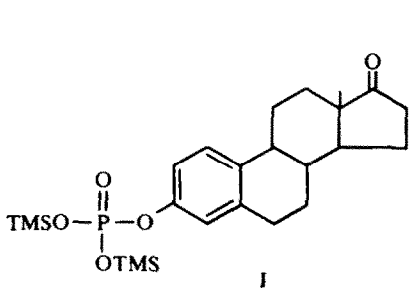
The compounds studied in this investigation were the TMS derivatives of 3-hydroxy-1.3.5(10)-estratrien-17-one-3-phosphate (I). 3,17β-dihydroxy-1.3.5(10)-estratriene-17β-phosphate (II). 3.17β-dihydroxy-1.3.5(10)-estratriene-3-phosphate (III). 3.17β-dihydroxy-1.3.5(10)-estratriene-3.17β-diphosphate (IV). 11β.17α-21.trihydroxypregna-1.4-diene-3.20-dione-3.20-dimethyloxime-21-phosphate (V). 11β.17α.21-trihydroxypregn-4-ene-3.20-dione-3.20-dimethyloxime-21-phosphate (VI). Their mass spectra are shown in Figs 1–6.

Compound I contains no trimethylsilyl group other than the two on the phosphate moiety and consequently would not be expected to form ions of types **a**, **b** and **d**. Compounds II and III on the other hand contain an additional trimethylsilyl group, but as these functional groups are placed at the two extreme ends of the steroid molecule, little interaction to form ions **a**, **b** and **d** should occur if stereochemistry plays a significant role in the formation of these ions. In addition, the diphosphate derivative (IV) offers the possibility for studying the migration of intact trimethylsilylphosphate groups to give rearrangement ions of the pyrophosphate type observed as fragments in the mass spectra of the TMS derivatives of glycerol diphosphates.[13] Finally, in the pregnene derivatives (V and VI) the positional orientation is favorable for trimethylsilylphosphate and trimethylsilyl group interactions and thus a high abundance of rearrangement ions would be expected to occur.

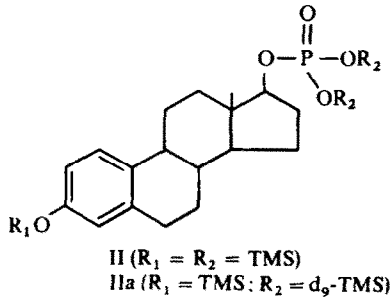
Rearrangement ions

A feature of the mass spectra of compounds I–VI is the retention of the charge with the phosphate moiety, a situation typical of the TMS derivatives of sugar phosphates. The high abundance of the resulting characteristic phosphorus-containing ions is thought to be a direct reflection of the resonance stabilization possible when all the phosphate oxygens are substituted. For this reason cyclic structures have been depicted for ions **d–f**, although evidence has been presented to support an additional open chain structure with charge localization on the silicon atom.[10]

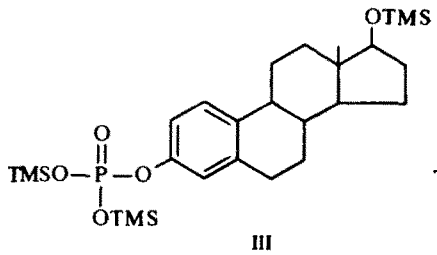
The ions at *m/e* 315 (**b**) and 299 (**d**) which involve the migration of a TMS group to the phosphate moiety are among the most abundant in the mass spectra of the trimethylsilyl derivatives of sugar phosphates. Formation of ion **b** involves both hydrogen and TMS transfer to the phosphate moiety. The presence of the appropriate



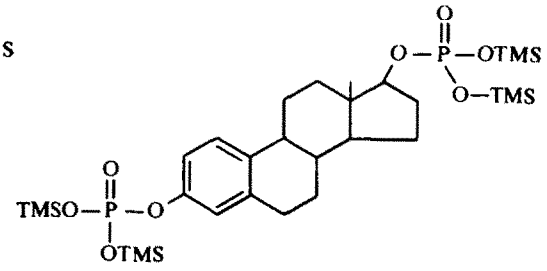
I



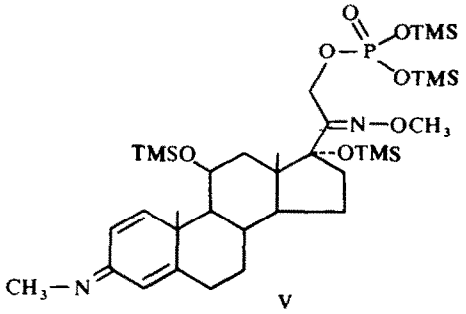
II ($R_1 = R_2 = TMS$)
IIa ($R_1 = TMS$; $R_2 = d_9$-TMS)



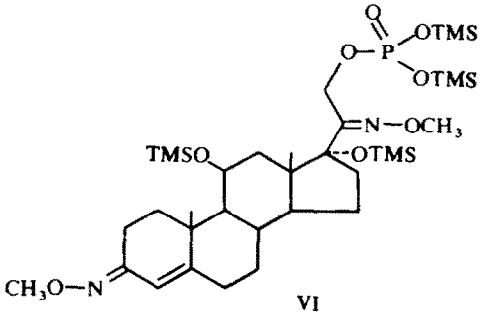
III



IV



V



VI

metastables show that ion **d** is predominantly formed by elimination of methane from **b**. as well as by rearrangement of the M-15 ion.[8, 13] As expected. the wide separation of the trimethylsilyloxy and trimethylsilylphosphate groups in compounds II and III results in the low abundance of the rearrangement ions **b** and **d** in their spectra. This shows that the positioning of the interacting groups is important for the favorable production of these ions. A small amount of interaction between the isolated TMS and trimethylsilylphosphate groups is evident (**b**, 13%, 1% Σ_{40} and **d**, 12%, 1% Σ_{40} in II; **b**, 4·5%, 0·3% Σ_{40} and **d**, 8·0%, 0·6% Σ_{40} in III).* This may be the result of ring cleavage prior to interaction. A considerable increase in the abundance of the ions arising from a TMS group transfer to a trimethylsilylphosphate moiety can be seen in the spectrum of the diphosphate derivative IV (**b**, 25%, 1·2% Σ_{40} and **d**, 60%, 2·7% Σ_{40}). Here, in addition to the presence of two trimethylsilylphosphate moieties, the phosphate TMS groups are not attached directly to the steroid nucleus and should be expected to interact to a greater extent. Ions of mass 299 and 315 could conceivably

* All intensities are normalized to the base peak. The usually highly abundant ions of *m/e* 73 and 75 were disregarded when assigning the base peak because of their lack of reproducibility.

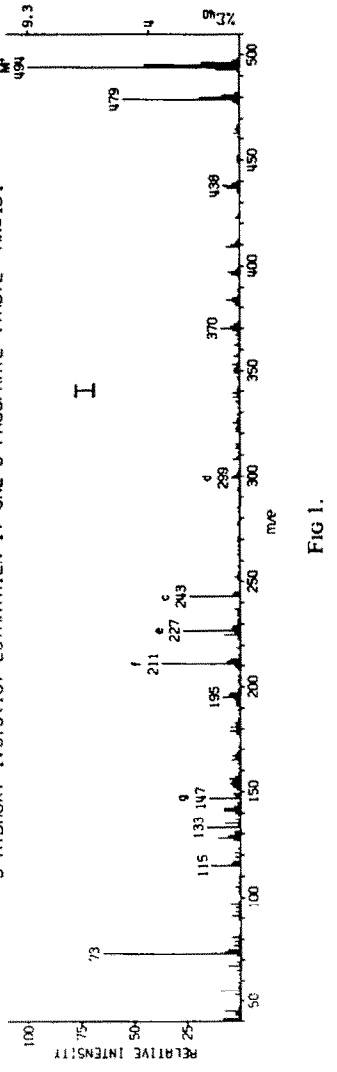

3-HYDROXY-1,3,5(10)-ESTRATRIEN-17-ONE 3-PHOSPHATE (TMS)2 MW=494
I
M+ 494
479
73
115 133 147
195
211
227
243
289
370
m/e
RELATIVE INTENSITY

FIG 1.

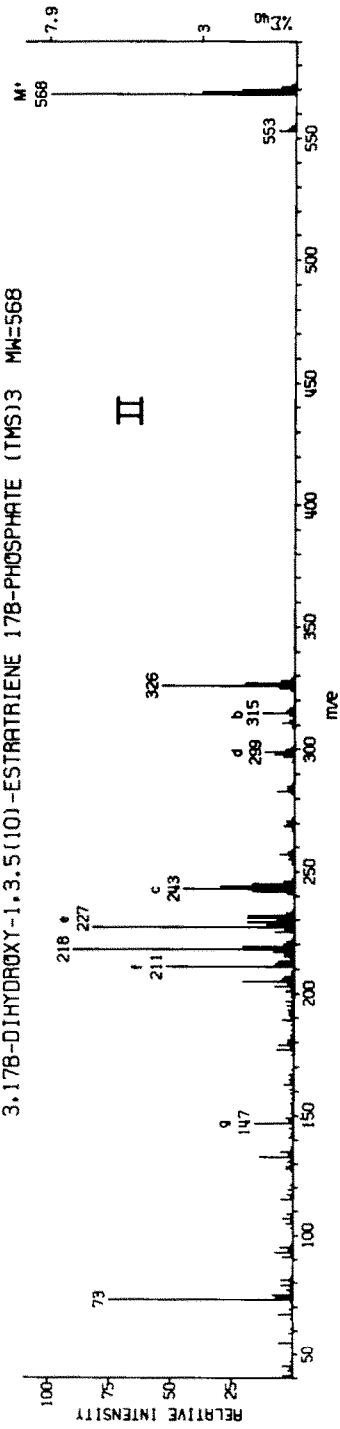

3,17B-DIHYDROXY-1,3,5(10)-ESTRATRIENE 17B-PHOSPHATE (TMS)3 MW=568
II
M+ 568
553
326
315
289
243
227
218
211
147
73
m/e
RELATIVE INTENSITY

FIG 2.

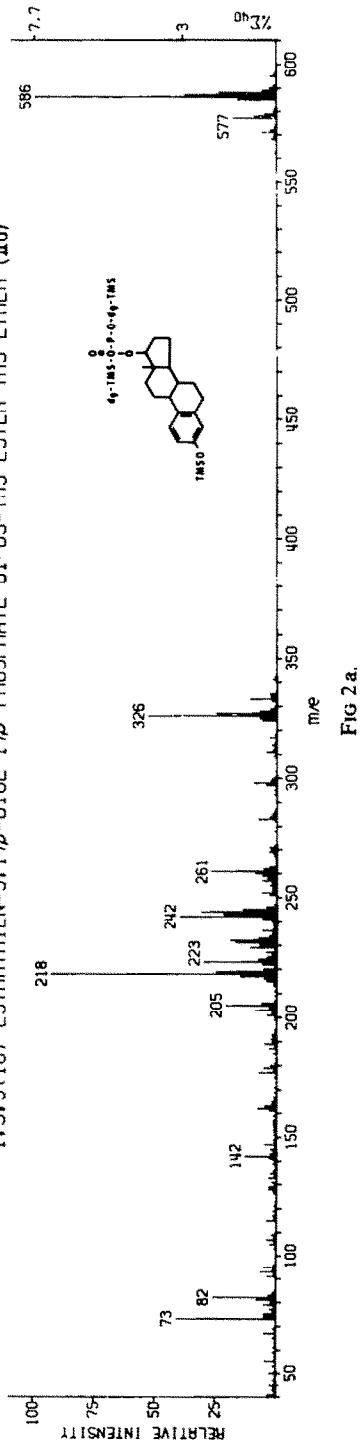

1.3.5(10)-ESTRATRIEN-3.17β-DIOL 17β-PHOSPHATE DI-OS-TMS ESTER TMS ETHER (IIa)
RELATIVE INTENSITY
m/e
586
577
326
261
242
223
218
205
142
82
73

FIG 2a.

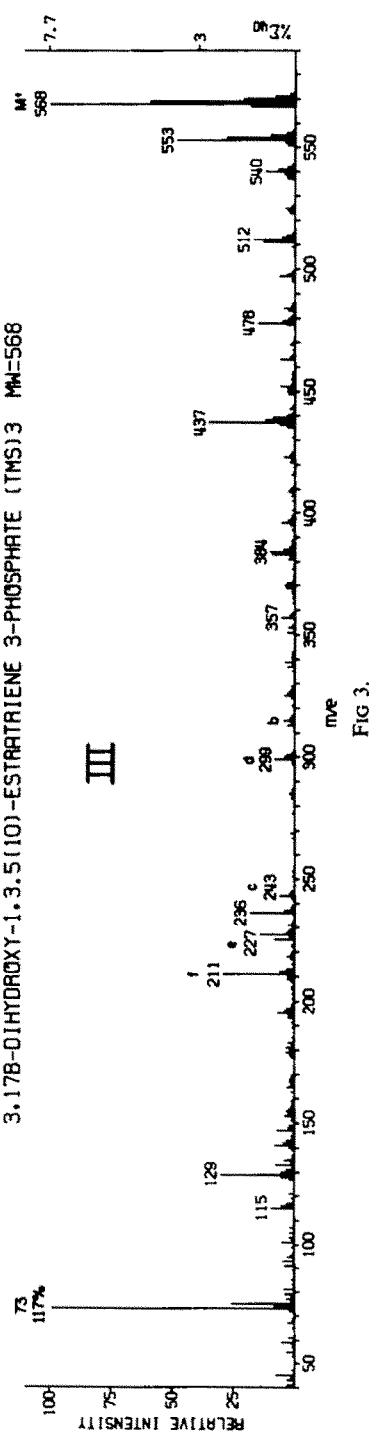

3.17B-DIHYDROXY-1.3.5(10)-ESTRATRIENE 3-PHOSPHATE (TMS)3 MW=568
III
RELATIVE INTENSITY
m/e
568
553
437
384
357
298
243
236
227
211
129
115
73

FIG 3.

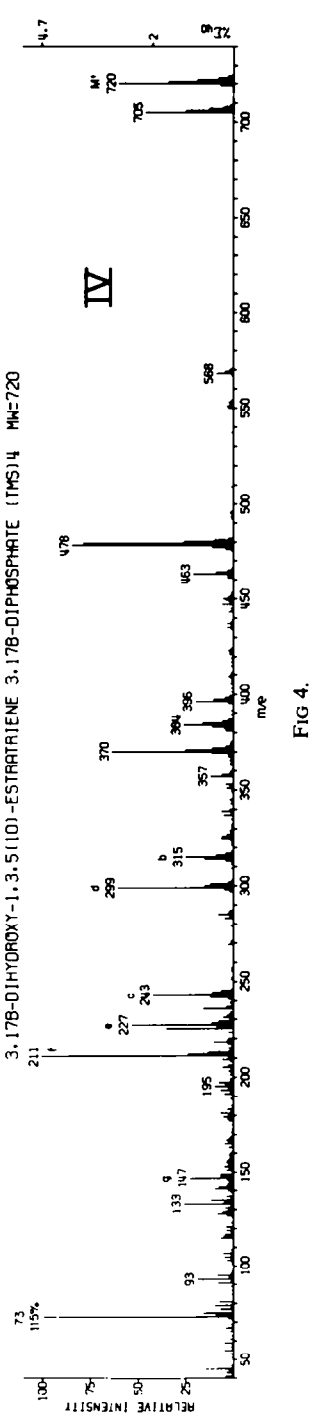

3.17B-DIHYDROXY-1.3.5(10)-ESTRATRIENE 3.17B-DIPHOSPHATE (TMS)4 MW=720
IV
RELATIVE INTENSITY
m/e

FIG 4.

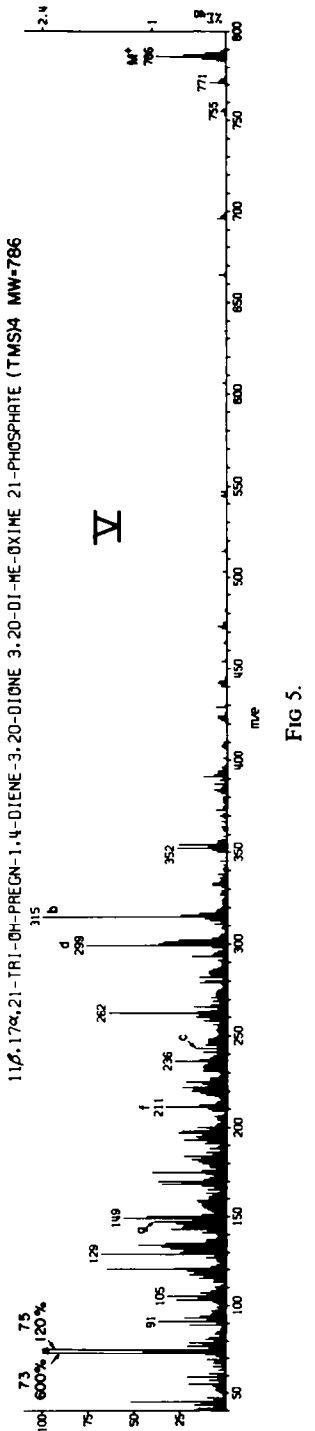

11β.17α.21-TRI-OH-PREGN-1.4-DIENE-3.20-DIONE 3.20-DI-ME-OXIME 21-PHOSPHATE (TMS)4 MW=786
V
RELATIVE INTENSITY
m/e

FIG 5.

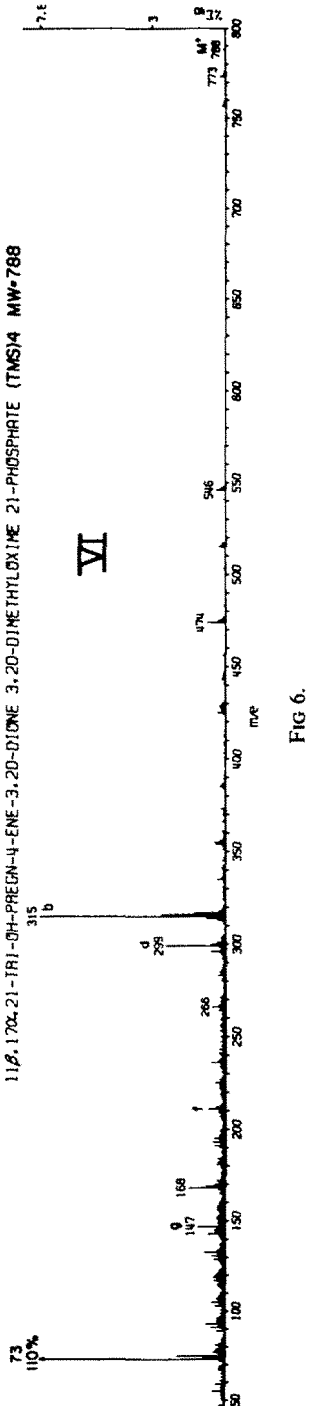

11β,17α,21-TRI-OH-PREGN-4-ENE-3,20-DIONE 3,20-DIMETHYLOXIME 21-PHOSPHATE (TMS)4 MW=788
IV
RELATIVE INTENSITY
m/e
73
110%
315
b
299
d
168
147
946
774
773
788

FIG 6.

arise from rearrangement of other fragment ions, but this is thought unlikely in view of earlier studies which indicated that TMS rearrangement ions are usually formed prior to ring cleavage in steroids.[12]

This situation, whereby the rigid steroid nucleus precludes the favorable migration of TMS groups, is reversed in the pregnene derivatives (V and VI) where the ethereal trimethylsilyl groups are closer to the phosphate moiety and favorable interaction can occur. Migration of either the 11β or the 17α trimethylsilyloxy group may occur, but it is not possible to distinguish which is preferential on the basis of the present data. The rearrangement ion at *m/e* 315 (**b**) is now the base peak in the spectra of both compounds. The previously observed ion at *m/e* 387 (**a**) produced by double TMS migration[2,8] is of very low abundance and this may be due, in part, to the steric effect of the 18-angular Me group which prevents the favorable migration of the 11β-TMS group to the phosphate moiety.

No ion arising from TMS migration to the phosphate moiety should occur in the spectrum of the TMS derivative of the hydroxy ketone (I). The weak peaks which are observed at *m/e* 315, 314, and 299 are probably due to partial thermal dissociation of the compound on the chromatographic column[13] and subsequent formation of tris-trimethylsilylphosphate,[14] or due to an ion molecule reaction product.[9,10]

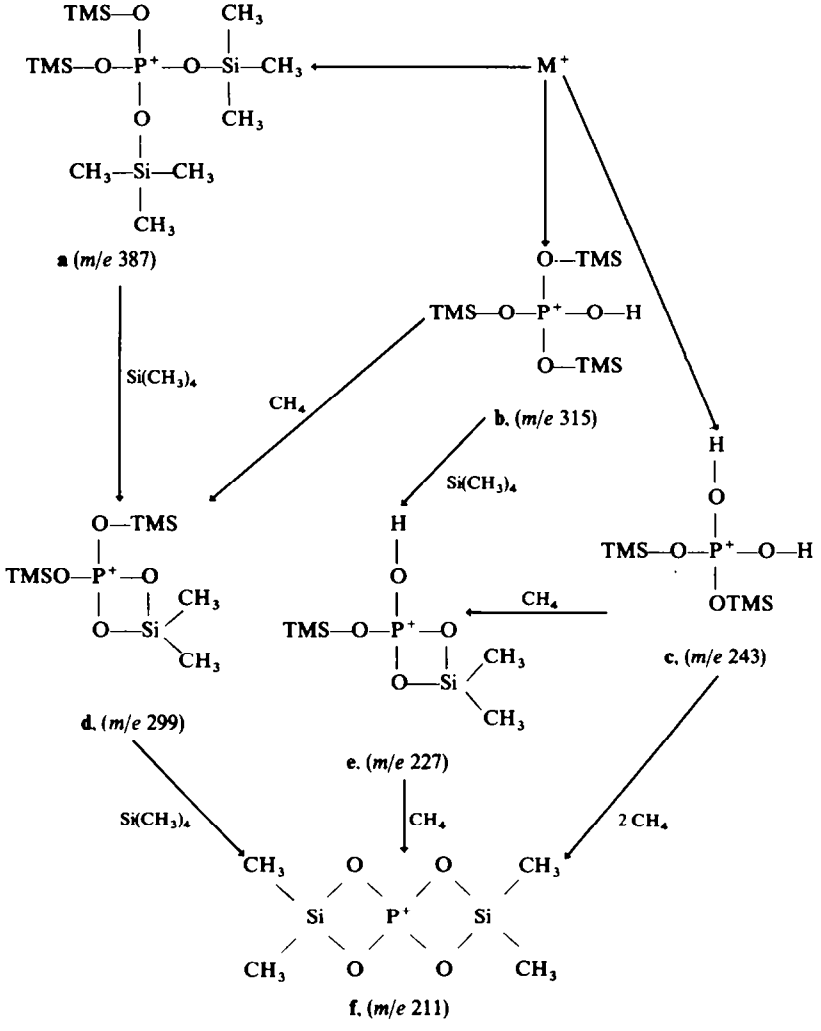
In the absence of adjacent TMS groups, rearrangement ions requiring hydrogen transfer to the phosphate moiety become increasingly prominent. The relative abundance of ion **e** (*m/e* 227) is 27% (2·3% Σ_{40}) in I, 82% (6·5% Σ_{40}) in II, 14% (1·2% Σ_{40}) in III, and 53% (2·5% Σ_{40}) in IV. By contrast the generally low abundance of **e** in sugar phosphates may be noted, as well as its virtual absence in the spectra of the pregnenes V and VI (9%, 0·2% Σ_{40} and 3%, 0·2% Σ_{40} respectively). A double hydrogen transfer is responsible for the formation of **c** (*m/e* 243). This ion is again of high abundance in the spectra of the estriene derivatives II and IV (45%, 3·6% Σ_{40} and 42%, 2·0% Σ_{40} respectively). The shortage of available hydrogens within close proximity to the phosphate group of these compounds is presumably responsible for the low abundance of **c** in the spectrum of the 3-phosphate derivative III (6%, 0·5% Σ_{40}). In view of this, the relative abundance of **c** in the spectrum of the 3-phosphate derivative (I) is surprisingly high (24%, 2·2% Σ_{40}) and may be due to different fragmentation modes in I and III. This may be induced by the variation in the substitution of the 17-position, resulting in a fragment ion, a hydrogen of which may be favorably disposed to transfer to the phosphate moiety. The pregnene derivatives (V and VI) in which ethereal TMS groups are in favorable positions to migrate to the trimethylsilylphosphate group, show the anticipated low abundance of ion **c** (17%, 0·4% Σ_{40} and 3·5%, 0·2% Σ_{40} respectively) analogous to the low abundance of ion **e** (*m/e* 227) which involved a single hydrogen transfer.

Ion **f** (*m/e* 211) is predominantly formed as a fragment from the rearrangement ions at *m/e* 227, 243 and 299 by loss of the elements of methane, C_2H_8, and the elements of tetramethylsilane respectively, as shown in Scheme 1. Metastable transitions have been observed for the first two pathways in the systems under discussion and for the third pathway in analogous systems.[13] Other data have provided evidence that the transition *m/e* 227 → *m/e* 211 represents the main pathway for the formation of the latter ion.[2,13] Its relative abundance is well above 25% of the base peak in the mass spectra of the estriene derivatives I–IV but is very low in the pregnene phosphates V and VI. This is similar to the variation of the relative abundance of the ions at *m/e* 227

and 243 in the spectra of I–IV and suggests that in these compounds the transitions **c** → **f** and **e** → **f** are the predominant routes for the formation of *m/e* 211 rather than the transition **d** → **f**. This was further supported by selectively labeling the phosphate TMS groups with deuterium.[13]

The ubiquitous trimethylsilyl rearrangement ion of mass 147 (**g**) is present in all compounds I–IV. Selective labeling[8] with perdeuterio-TMS groups showed that in the spectra of the monophosphates (II and III) **g** was formed exclusively by the interaction of the phosphate-TMS moieties. This is demonstrated by the mass spectrum (Fig 2a) of the selectively labeled trimethylsilyl derivative of 3,17β-dihydroxy-1.3.5(10)-estratriene-17β-phosphate (IIa) in which the phosphate TMS groups have been replaced by d_9-TMS (85% replacement). The observed 15 *amu* shift of *m/e* 147 to *m/e* 162 indicates no migration of the ethereal-TMS groups to the phosphate moiety. On the other hand, in the pregnenes V and VI some interaction was observed

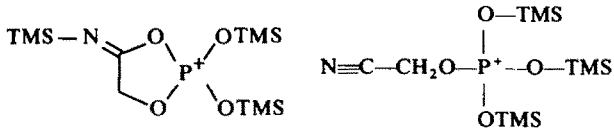
SCHEME 1



between the phosphate and ethereal-TMS groups. However ion **g** was again predominantly formed by the interaction of the phosphate-TMS groups and, consistent with previous observations,[12] there was little interaction between the 17α- and 11β-trimethylsilyloxy groups.

As discussed earlier the diphosphate derivative (IV) offers the possibility for the study of ions involving the rearrangement and interaction of two trimethylsilylphosphate groups. Ions of this type have been observed in moderate amounts in the TMS-derivatives of glycerol diphosphates,[13] but the separation of the two functional groups in IV prohibits their favorable interaction and consequently none of these ions are observed in its mass spectrum.

An interesting rearrangement ion (*m/e* 354) is present in the mass spectra of the pregnene derivatives V and VI (26% and 6% respectively) but not in the spectra of methyloxime derivatives of trimethylsilyl sugar phosphates. It exhibited a 27 *amu* shift in the spectra of the d_9-TMS analogues and an 18 *amu* shift upon selective d_9-TMS labeling of the trimethylsilylphosphate group, indicating that it contained the latter group and an ethereal TMS moiety. Structures **h** and **i** are thought to be the most likely for this ion. Structure **h** is the nitrogen analogue of the oxygen-containing ion at *m/e* 357 reported in the mass spectra of TMS derivatives of sugar



h (*m/e* 354) **i** (*m/e* 354)

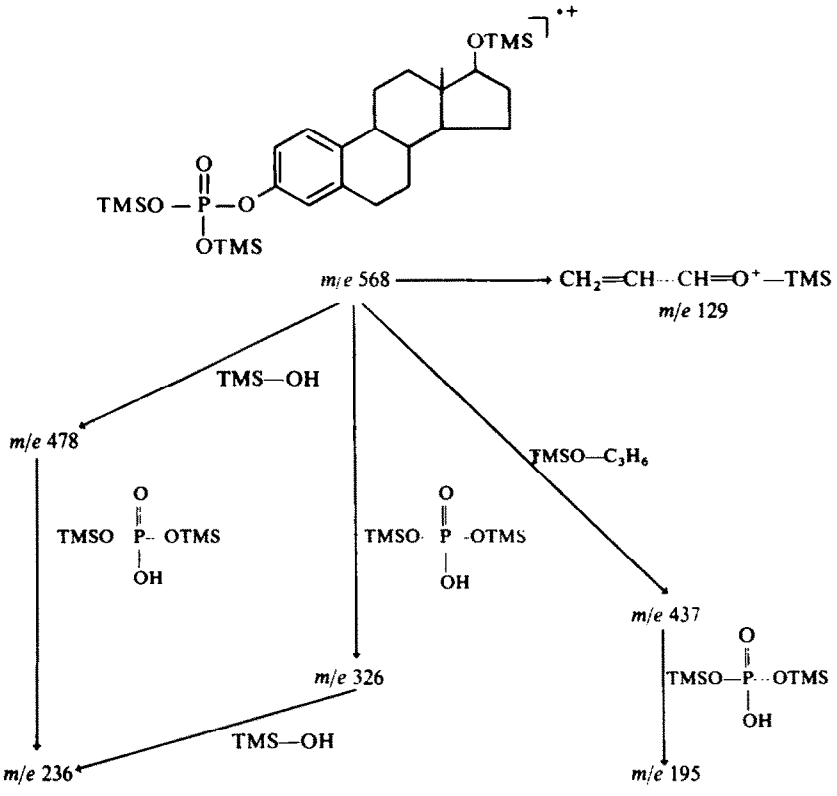
phosphates.[2] Its formation involves TMS transfer to a nitrogen atom whereas in **i** the TMS moiety has migrated to the phosphate group. The latter process has been observed repeatedly in compounds of this type.

Other significant fragment ions

Examination of the mass spectra of the estrienes (I–IV) shows that aside from the characteristic trimethylsilylphosphate-containing ions (*e.g.*, **a–f**), the other major fragment ions are typical of trimethylsilyl derivatives of hydroxy-estrogens.[15] The molecular ions are of high abundance unlike those of the TMS derivatives of sugar phosphates.[2]

The trimethylsilyl and acetyl derivatives of steroidal alcohols exhibit characteristic peaks due to the elimination of trimethylsilanol and acetic acid respectively.[16] Various elimination processes of this type have been observed in the mass spectra of the estrienes (I–IV). For example, Scheme 2 summarizes the major elimination processes observed in compound III. Similarly, in compounds II and IV a favorable elimination of the intact 17β-trimethylsilylphosphate group is observed accompanied by hydrogen abstraction to give the ions at *m/e* 326 in II and *m/e* 478 in IV. Simple cleavage of the trimethylsilylphosphate groups is also observed, but the resulting ions (*m/e* 327 in II and *m/e* 479 in IV) are of lower abundance than the corresponding ions produced by elimination of hydrogen-*bis*-trimethylsilylphosphate. The mass spectra of perdeuterio-TMS derivatives[17] confirmed the identity of these ions. The occurrence of this elimination process was further verified by selectively replacing

SCHEME 2

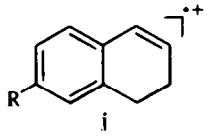


the phosphate-TMS groups with d_9-TMS while leaving all other TMS moieties unlabeled. The corresponding elimination of the trimethylsilylphosphate moiety in the 3-phosphate derivatives I and III was much less favorable presumably because of the attachment of the phosphate group to the aromatic ring. Elimination of trimethylsilanol from the molecular ion to give *m/e* 478 is observed in III and selective labeling with d_9-TMS confirmed that it involved almost exclusively the 17β-trimethylsilyloxy group. The process of elimination of the 17β-group in the 3-phosphate derivatives (III and IV) is followed by ejection of the trimethylsilylphosphate group from the 3-position accompanied by hydrogen abstraction to give the ion at *m/e* 236.

The characteristic loss of the D-ring together with hydrogen abstraction, typical of 17-substituted steroids,[18,19] is observed in varying degrees in the mass spectra of the estrienes (I, III and IV) to give the ion at *m/e* 437. Some direct cleavage without hydrogen transfer to give *m/e* 438 is also observed in the spectrum of the 17-oxo derivative (I). Elimination of the TMS-phosphate moiety, as discussed above, from *m/e* 437 gives the ion at *m/e* 195.

Loss of the C- and D-rings is a typical fragmentation process in estrogen derivatives.[15] It accounts for the peaks at *m/e* 370 in the spectra of the 3-phosphate derivatives (I, III and IV) and *m/e* 218 in II to give the ion **j**.

The most notable ions in the mass spectra of the pregnene derivatives (V and VI) are the trimethylsilylphosphate-containing rearrangement ions of mass 299 and 315



i

R = TMSO—P(=O)(—O)—OTMS in I. III. IV (*m/e* 370)

R = OTMS in II (*m/e* 218)

(Figs 5 and 6). Most of the other ions are typical of trimethylsilyl and methyloxime derivatives of steroids. For example, loss of the hydrogen-*bis*-trimethylsilylphosphate moiety gives rise to the low intensity peaks at *m/e* 544 and 546 in the spectra of V and VI respectively. An analogous process involving a TMS rather than a hydrogen abstraction results in the elimination of *tris*-trimethylsilylphosphate with the production of an ion at *m/e* 474 in the mass spectrum of VI. Furthermore, both compounds exhibit the characteristic successive losses of 90 *amu* due to elimination of trimethylsilanol (*e.g.*, *m/e* 786 → *m/e* 696 → *m/e* 606 in V). Losses of 31 *amu* ($\cdot OCH_3$) from the molecular ion, a process frequently observed in methyloxime derivatives of keto-steroids, can be seen in the mass spectra of V and VI.

Relatively abundant ions arising from retention of the charge with the steroid nucleus are present in the mass spectra of both V and VI. Selective labeling with perdeuteriotrimethylsilyl groups showed that the ions at *m/e* 352 and *m/e* 168 in V and VI respectively are fragments containing one ethereal-TMS group. The abundant ion at *m/e* 262 in V does not shift upon labeling with d_9-TMS and hence contains only parts of the steroid nucleus.

CONCLUSIONS

The data presented above indicate that TMS group migrations in phosphate derivatives of rigid structure are specific processes and are dependent on the positioning of the interacting groups. The abundance of the ions formed in this way is dependent on the separation and isolation of the functional groups concerned. Rearrangement ions of mass 387, 315 and 299 which are highly abundant in sugar phosphates are virtually absent or of low abundance in cases where the interacting groups are well separated. In these cases, because of the high stability of phosphate ions carrying a substituent on each oxygen, ions involving hydrogen migrations become increasingly predominant.

EXPERIMENTAL

Samples of steroidal phosphates were commercially available as their sodium salts from Steraloids, Inc. (I–V) and Sigma Chemical Co. (VI). Trimethylsilyl derivatives of I–IV (1 mg) were prepared by their reaction with *bis*-trimethylsilyltrifluoracetamide (BSTFA, 0·1 ml) and trimethylchlorosilane (TMCS, 0·5 ml) in MeCN (0·1 ml), at 80° for 30 min. Derivatives of V and VI were prepared as follows: the steroid

(1 mg) was reacted with an excess of hydroxylamine hydrochloride in pyridine (0·2 ml) at 60° overnight. The pyridine was removed under N_2 and the trimethylsilyl derivatives were prepared by dissolving the methyloxime in MeCN (0·1 ml), BSTFA (0·1 ml), trimethylsilylimidazole (TMSI, 0·1 ml) and TMCS (0·05 ml) and heating the resulting mixture at 150° for 5 hr in a sealed tube.[20] For perdeuterio-TMS analogues, labeled TMS reagents were employed.[17] Selective labeling was performed by saturating the GLC column with 10 µl of a 20:1 mixture of d_{18}-BSA:d_9-TMCS and injecting the unlabeled TMS derivative of the steroid sample. This was immediately followed by injection of an additional 10 µl of the d_{18}-BSA/d_9-TMCS mixture. It was thus possible to replace the more labile phosphate-TMS groups with d_9-TMS.

All mass spectra were obtained at 70 eV ionizing energy using an LKB-9000 mass spectrometer. The accelerating voltage was 3·5 KV and the source temperature 310°. Samples were introduced through the gas chromatographic inlet using a 6 ft 1% OV-17 column.

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