

[Chem. Pharm. Bull.]
34(8)3298—3305(1986)]

Diethylhydrogensilyl Cyclic Diethylsilylene Derivatives in Gas Chromatography-Mass Spectrometry of Hydroxylated Steroids. II. Pregnanes with a Hydroxylated 17 β -Side-Chain

MASATAKA ISHIBASHI,*^a MASAHIRO ITOH,^a KOUWA YAMASHITA,^a
HIROSHI MIYAZAKI,^b and HISAO NAKATA^c

Research Laboratories, Pharmaceutical Group, Nippon Kayaku Co.,^a Shimo, Kita-ku, Tokyo 115, Japan,
Corporate Planning Office, Nippon Kayaku Co.,^b Fujimi, Chiyoda-ku, Tokyo 102, Japan
and Department of Chemistry, Aichi Kyoiku University,^c
Igaya, Kariya, Aichi 448, Japan

(Received February 6, 1986)

The gas chromatographic-mass spectrometric properties of diethylhydrogensilyl cyclic diethylsilylene derivatives of hydroxypregnanes were studied. Pregnanes with a sterically hindered 11 β -hydroxyl group were smoothly silylated with a new silylating agent, *N,O*-bis(diethylhydrogensilyl)-trifluoroacetamide, under mild conditions. The mass spectra of these derivatives were characterized by the appearance of the intense molecular ion peak, which provides convenient and reliable confirmation of the molecular weight of these hydroxypregnanes. The most characteristic fragment ions were those at m/z 185 for 17,20-diols and at m/z 287 for 17,20,21-triols, which were produced by cleavage at the C(13)–C(17) and C(15)–C(16) bonds with hydrogen atom transfer. This indicates that all of the major fragmentations are directed by the cyclic diethylsilylene group. Another fragmentation common to the spectra was the successive loss of diethylhydrogensilanol from the molecular ion. A remarkable difference in the appearance of peaks was observed between DEHS-DES derivatives of a 17 α ,20 α ,21-triol and its 20 β -isomer. Structures are proposed for some of these fragment ions.

Keywords—hydroxylated steroid; hydroxypregnane; derivatization; GC-MS; cyclic diethylsilylene derivative; mass spectrum; mass spectral fragmentation; DEHS-BSTFA

For gas chromatography-mass spectrometry (GC-MS) analysis of biologically important substances, it is necessary to convert them into volatile and thermally stable derivatives. Trimethylsilyl (TMS) ethers have been used extensively for separation, identification and quantitation purposes, especially by GC-MS. Recently, several other kinds of silyl ether derivatives have also been used for GC-MS analysis.^{1–8)}

It has been reported that steroidal alkyldimethylsilyl ether derivatives such as dimethyl-ethylsilyl,⁵⁾ dimethyl-*n*-propylsilyl (DMNPS),⁵⁾ dimethylisopropylsilyl⁶⁾ and *tert*-butyl-dimethylsilyl (TBDMS) ethers²⁾ and allyldimethylsilyl ethers³⁾ gave abundant $[M - \text{alkyl}]^+$ (or $[M - \text{allyl}]^+$) ions in the electron impact ionization (EI)-mode. These derivatives have found considerable application in selected ion monitoring of hydroxylated compounds such as steroids and prostaglandins. However, no silyl ether derivatives of hydroxypregnanes with sterically hindered 17 α -hydroxyl group could provide the above superior properties because of difficulties in derivatization without formation of by-products and because of the formation of the prominent fragment ion due to preferential fission of the C(17)–C(20) bond.^{3,5,6,9)} Recently, Andersson and Sjövall have investigated the GC-MS properties of the mixed TMS-TBDMS ether derivatives of steroids with a sterically hindered 17 α -hydroxyl group obtained by the use of intramolecular silylation reaction.¹⁰⁾ The mass spectral fragmentations of these TMS-TBDMS ethers were similar to those of the mixed TMS-DMNPS.⁵⁾

Anthony *et al.*¹¹⁾ reported the utility of alkyl- and arylboronic acids as derivatizing agents for the formation of cyclic boronate derivatives from bifunctional alcohols. One disadvantage of these derivatives is that the remaining hydroxyl group after treatment with alkylboronic acids requires an additional derivatizing agent. On the other hand, the 3-acetoxydimethylsilyl ether-dimethylsilylene (DMS) derivative of 5 β -pregnane-3 α ,17 α ,20 β -triol gave a mass spectrum (MS) including an intense molecular ion.¹²⁾ However, there has been little application of this DMS derivative for GC-MS analysis because of its hydrolytic instability.

In the previous paper,¹³⁾ we reported the GC-MS properties of diethylhydrogensilyl (DEHS) and diethylhydrogensilyl cyclic diethylsilylene (DEHS-DES) derivatives prepared by the use of a new silylating agent, *N*, *O*-bis(diethylhydrogensilyl)trifluoroacetamide (DEHS-BSTFA), from twenty hydroxysteroids including a few pregnane-3,17,20-triols. The MS of these DEHS-DES derivatives were characterized by their strong molecular ion peak, which clearly suggests that they are useful not only for identification but also for microanalysis by GC-MS. This paper deals with the GC and GC-MS properties of DEHS-DES derivatives of hydroxypregnanes with 17,20-diol, 20,21-diol and 17,20,21-triol structures.

Experimental

Gas Chromatography (GC)—A Shimadzu GC-7A gas chromatograph (Shimadzu Seisakusho Co., Kyoto, Japan) equipped with a flame ionization detector and a Van den Berg's solventless injector¹⁴⁾ was employed. The column was a 25 m fused silica capillary column cross linked with methylsilicon (Ultra 1, Hewlett Packard Co., PA, U.S.A.). The temperature of the column oven was maintained at 250–290 °C. The carrier gas was helium at a linear velocity of 25 cm/s. The temperature of the injection port and detector was kept at 300 °C.

GC-MS—A VG Micromass ZAB-HF mass spectrometer (VG Analytical Ltd., Manchester, U.K.) interfaced to a Shimadzu GC-9A gas chromatograph (Shimadzu Seisakusho Co.) with a solventless injector and a DS-2035 data processing system (VG Analytical Ltd.) was employed. The capillary column was directly introduced into the mass spectrometer source. GC conditions were the same as above. The carrier gas was helium at a velocity of 30 cm/s. The temperature of the injector port and transfer line was kept at 290 °C and that of the ion source at 250 °C. The ionization energy and trap current were 70 eV and 200 μ A, respectively. The accelerating voltage was 8 kV.

The MS of each of the pregnane derivatives was recorded by repeated scanning (2.0 s/decade) in the range of m/z 850-90 (cycle time 3 s) with a dynamic resolution of 2000. High resolution MS was carried out under the same GC-MS conditions as used in the low resolution mode. The mass range between m/z 800-90 was scanned at 3 s/decade (cycle time 4 s) with a dynamic resolution of 5000. The linked scanning was achieved throughout standard hardware, and the data system was used to control repeated scanning at constant B/E with a total scan cycle of 5–6 s.

Samples and Reagents—The steroids used in this study were obtained commercially, from Steraloids Inc. (NH, U.S.A.) and Sigma Chemical Co. (MO, U.S.A.). 5 β -Pregnane-3 α , 17 α , 20 β -triol was kindly donated by Dr. A. Tsuji. DEHS-BSTFA was synthesized in our laboratory as previously reported.¹³⁾ Pyridine was of amino acid analytical grade and was used without further purification. *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide (TBDMs-MSTFA) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan).

Derivatization—DEHS-BSTFA (50 μ l) was added to a solution (100 μ l) of a steroid sample (0.1–0.2 mg) in pyridine. The solution was allowed to stand for 30 min to 1 h at room temperature. The resulting solution was used directly for GC and GC-MS analyses.

Results and Discussion

Gas Chromatography (GC)

In this study, ten hydroxypregnanes with 17,20-diol, 20,21-diol and 17,20,21-triol moieties were examined. The corresponding DEHS-DES derivatives of hydroxypregnanes were obtained by using DEHS-BSTFA as a silylating agent. Figure 1 shows the gas chromatograms of the reaction products of 5 β -pregnane-3 α ,17 α ,20 β -triol (**2**), 5 β -pregnane-3 α ,20 β ,21-triol (**3**), 5 β -pregnane-3 α ,11 β ,17 α ,20 β -tetraol (**5**), 5 β -pregnane-3 α ,17 α ,20 α ,21-tetraol (**7**) and 5 β -pregnane-3 α ,11 β ,17 α ,20 β ,21-pentaol (**10**) as representative hydroxypregnanes. All steroids were readily converted into the expected derivatives under mild conditions, such as at room temperature within 1 h, and each of the resulting products ex-

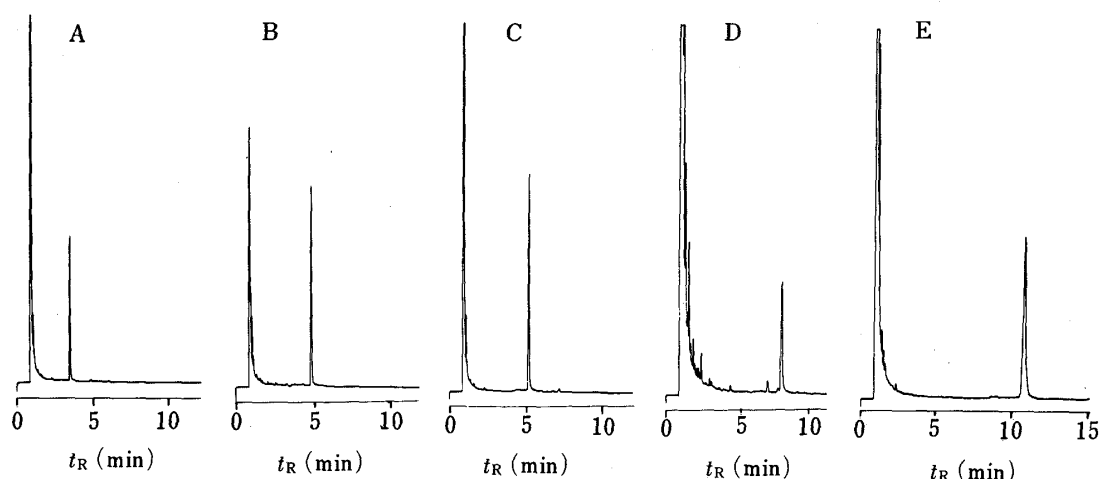


Fig. 1. Gas Chromatograms of the DEHS-BSTFA Reaction Products

A: 5β -pregnane- $3\alpha,17\alpha,20\beta$ -triol (2), B: 5β -pregnane- $3\alpha,20\beta,21$ -triol (3), C: 5β -pregnane- $3\alpha,11\beta,17\alpha,20\beta$ -tetraol (5), D: 5β -pregnane- $3\alpha,17\alpha,20\alpha,21$ -tetraol (7), E: 5β -pregnane- $3\alpha,11\beta,17\alpha,20\beta,21$ -pentaol (10).

hibited a well shaped single peak, except for pregnanes with a $17\alpha,20\alpha,21$ -triol moiety. The reaction product of each of 7 and 5β -pregnane- $3\alpha,11\beta,17\alpha,20\alpha,21$ -pentaol (9, GC not shown) gave a well-resolved doublet consisting of a major (>90%) and a minor component. Pregnanes with a $17\alpha,20\beta,21$ -triol, 5β -pregnane- $3\alpha,17\alpha,20\beta,21$ -tetraol (8, GC not shown) and 10 were silylated quantitatively to their respective derivatives without formation of by-products.

Table I shows the methylene unit (MU)-values of the DEHS-DES derivatives of hydroxypregnanes studied. When the MU-values of four pairs of epimeric 20-hydroxy isomers were compared, the values of the α -isomers were about 0.4 to 0.6 larger than those of the β -isomers except for the pair of 7 and its 20β -isomer (8). The epimeric hydroxyl group at C-20 had a significant effect on the separation of isomers, and epimeric isomers were easily resolved under the above mentioned GC-conditions.

The sterically hindered 11β -hydroxyl group could not be silylated by the use of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), whereas the present DEHS-BSTFA easily introduced the thermally stable 11β -diethylhydrogensilyloxy (DEHSO) group. Steric factors of the reagent seem to be important in regulating the extent of silylation, and the approach of the silylating agent to a hydroxyl group is considered to be a key step of the reaction. As the DEHS group has a different geometry from the TMS group and seems to be less bulky, the steric factor in the case of the DEHS group may be smaller. Thus, the smaller DEHS group may be able to take a more favorable S_N2 transition state in the silylation reaction, so that the silylation of the 11β -hydroxyl group with DEHS-BSTFA can take place more smoothly than with BSTFA.

Mass Spectrometry (MS)

All pregnanes with vicinal diols and vicinal triols in the 17β -side-chain were easily converted to their DES derivatives in the same manner as cyclic boronate derivatives.^{11,12,15} The MS of DEHS-DES derivatives of vicinal diols were very simple, while those of vicinal triols gave rather complicated fragmentation patterns that were ascribed to fission of ring D and the side-chain. Each of the spectra gave the molecular ion peak with high intensity, which enables us to confirm easily the molecular weight except for 3, where preferential cleavage of the C(17)-C(20) bond is involved. Table I lists salient features of the MS of the pregnanes studied.

TABLE I. Mass Spectral Data for the DEHS-DES Derivatives of Hydroxypregnanes

Compounds	GC data		MS data m/z (relative intensity)				Other ions
	MU value	Base peak	M^{+}	$[M - C_2H_5]^+$	$[M - DEHSOH]^+$		
1) 5 β -Pregnane- 3 α ,17 α ,20 α -triol	31.02	185 (100)	506 (28)	477 (-)	402 (13)	387 (3), 255 (8), 228 (6), 173 (10), 172 (10), 171 (15), 157 (5), 147 (15), 119 (7), 103 (11)	
2) 5 β -Pregnane 3 α ,17 α ,20 β -triol	30.62	185 (100)	506 (86)	477 (-)	402 (2)	255 (7), 173 (11), 172 (12), 171 (28), 157 (8), 147 (15), 119 (11), 107 (8), 105 (8), 103 (26)	
3) 5 β -Pregnane- 3 α ,20 β ,21-triol	32.57	145 (100)	506 (-)	477 (4)	402 (30)	282 (13), 269 (12), 255 (22), 171 (12), 161 (8), 144 (11), 117 (34), 107 (12), 105 (12), 103 (13)	
4) 5 β -Pregnane- 3 α ,11 β ,17 α ,20 α -tetraol	33.59	185 (100)	608 (21)	579 (-)	504 (23)	238 (18), 184 (16), 171 (25), 158 (15), 147 (35), 131 (17), 119 (16), 107 (15), 105 (20), 103 (77)	
5) 5 β -Pregnane- 3 α ,11 β ,17 α ,20 β -tetraol	33.00	185 (100)	608 (21)	579 (-)	504 (15)	238 (17), 184 (21), 171 (29), 158 (16), 147 (27), 131 (15), 119 (15), 107 (15), 105 (21), 103 (78)	
6) 5 β -Pregnane- 3 α ,11 β ,20 α ,21-tetraol	35.12	145 (100)	608 (17)	579 (1)	504 (5)	205 (25), 171 (19), 149 (26), 147 (17), 135 (18), 133 (15), 107 (21), 106 (18), 105 (16), 103 (34)	
7) 5 β -Pregnane- 3 α ,17 α ,20 α ,21-tetraol	34.84	205 (100)	608 (73)	579 (5)	504 (5)	491 (42), 463 (68), 287 (48), 257 (42), 255 (94), 245 (57), 175 (66), 171 (51), 147 (63), 107 (45)	
8) 5 β -Pregnane- 3 α ,17 α ,20 β ,21-tetraol	34.82	205 (100)	608 (80)	579 (2)	504 (5)	287 (68), 283 (44), 257 (47), 245 (80), 233 (34), 203 (37), 175 (62), 171 (43), 147 (56), 103 (78)	
9) 5 β -Pregnane- 3 α ,11 β ,17 α ,20 α ,21-pentaol	36.96	205 (100)	710 (52)	681 (2)	606 (24)	287 (48), 255 (46), 253 (32), 245 (30), 175 (32), 171 (38), 157 (34), 147 (37), 145 (35), 103 (69)	
10) 5 β -Pregnane- 3 α ,11 β ,17 α ,20 β ,21-pentaol	36.54	205 (100)	710 (56)	681 (1)	606 (21)	287 (53), 257 (31), 245 (33), 175 (40), 171 (33), 157 (36), 147 (44), 107 (35), 105 (38), 103 (87)	

Pregnanes with a 17,20-Diol Structure—Figure 2A shows the MS of the DEHS-DES derivatives of 5β -pregnane- $3\alpha,17\alpha,20\alpha$ -triol (1). It can be seen that the spectrum gave the molecular ion peak at m/z 506 with high intensity. The base peak at m/z 185, which was characteristic for the DES group of 17,20-diols, was assigned to correspond to the fragment ion of m/z 157 from the 17,20-DMS derivative¹²⁾ and that of m/z 124 from 17,20-methylboronates.^{12,16)} Elimination of DEHSOH from the molecular ion gave the ion of m/z 402 with moderate intensity. Loss of the C-21 methyl group from the molecular ion afforded an additional weak peak at m/z 491. A similar satellite peak of m/z 402 was also observed at m/z 387. The $[M - \text{CH}_3]^+$ ion further fragmented to the ion of m/z 171 by the cleavage of ring D, as was already proposed by Brooks *et al.*¹⁶⁾ for alkylboronates (Chart 1).

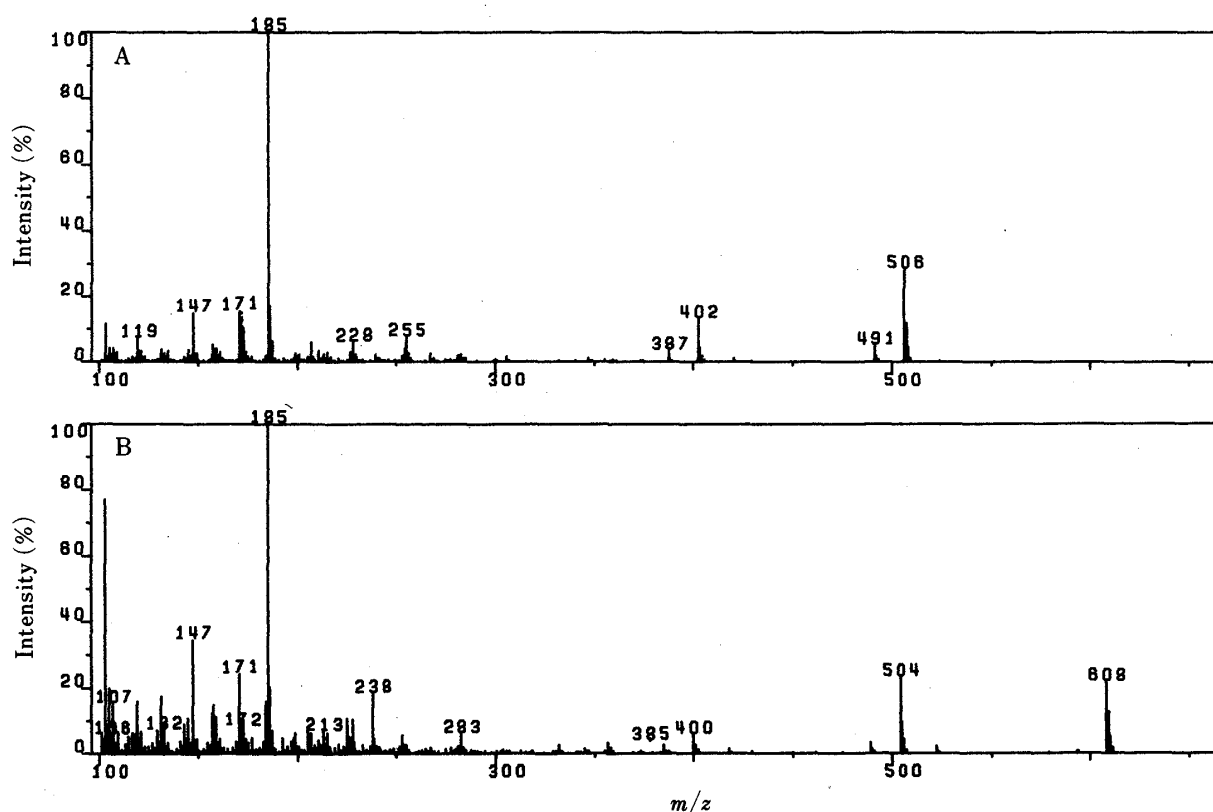


Fig. 2. Mass Spectra of the DEHS-DES Derivatives

A: 5β -pregnane- $3\alpha,17\alpha,20\alpha$ -triol (1), B: 5β -pregnane- $3\alpha,11\beta,17\alpha,20\alpha$ -tetraol (4).

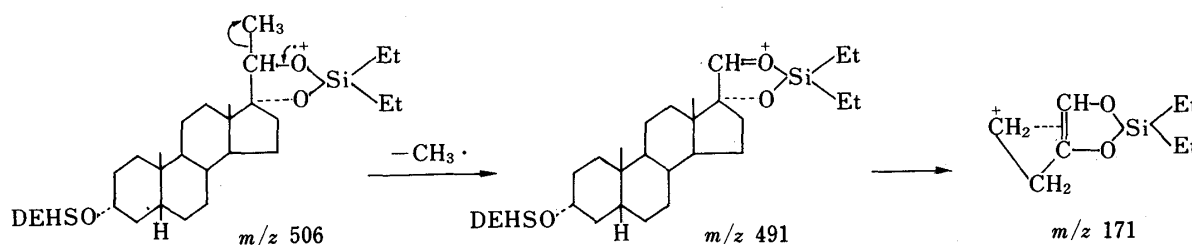
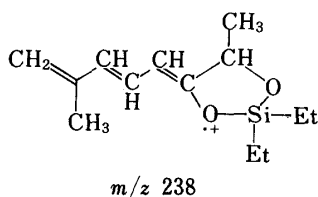


Chart 1

The MS of the DEHS-DES derivatives of 11β -hydroxylated pregnane-tetraols were similar to those of the triols except for the appearance of additional ions from the elimination of two molecules of DEHSOH. The ion of m/z 238 was characteristic in the spectra of DEHS-DES derivatives of 11β -hydroxylated pregnanes such as 5β -pregnane- $3\alpha,11\beta,17\alpha,20\alpha$ -tetraol (4) (Fig. 2B). Accurate mass measurement indicates that this ion has two oxygen atoms and

one silicon atom, suggesting that its structure may be as shown below. Formation of this ion may be due to a process similar to the fragmentation of the TMS ether derivatives of 11-hydroxylated steroids proposed by Vouros and Harvey.¹⁷⁾



Pregnanes with a 20,21-Diol Structure—The MS of 5 β -pregnane-3 α ,11 β ,20 α ,21-tetraol (**6**) 3,11-bis-DEHS-20,21-DES derivative gave a molecular ion peak with high intensity. Successive loss of DEHSOH from the molecular ion gave rise to the ions of $[M - (\text{DEHSOH})_n]^+$ at m/z 504 and 400. Elimination of a methyl group or an ethyl group from the molecular ion yielded another series of ions, from which the loss of DEHSOH was again observed. A characteristic fission of the C(17)–C(20) bond resulted in the formation of a base peak at m/z 145.

The MS of the DEHS-DES derivative of **3** exhibited a fragmentation pattern similar to that of **6**, as shown in Table I, although the molecular ion of **3** appeared with low abundance.

Pregnanes with a 17,20,21-Triol Structure—The MS of the DEHS-DES derivative of **8** gave a molecular ion peak with high intensity and a peak of $[M - \text{C}_2\text{H}_5]^+$ with very low intensity as shown in Figure 3B: the latter ion was presumably formed by elimination of an ethyl radical from one of the silyl groups. A weak peak of $[M - \text{DEHSOH}]^+$ at m/z 504 was also observed. Loss of the diethylhydrogensilyloxymethyl (CH_2ODEHS) radical from the

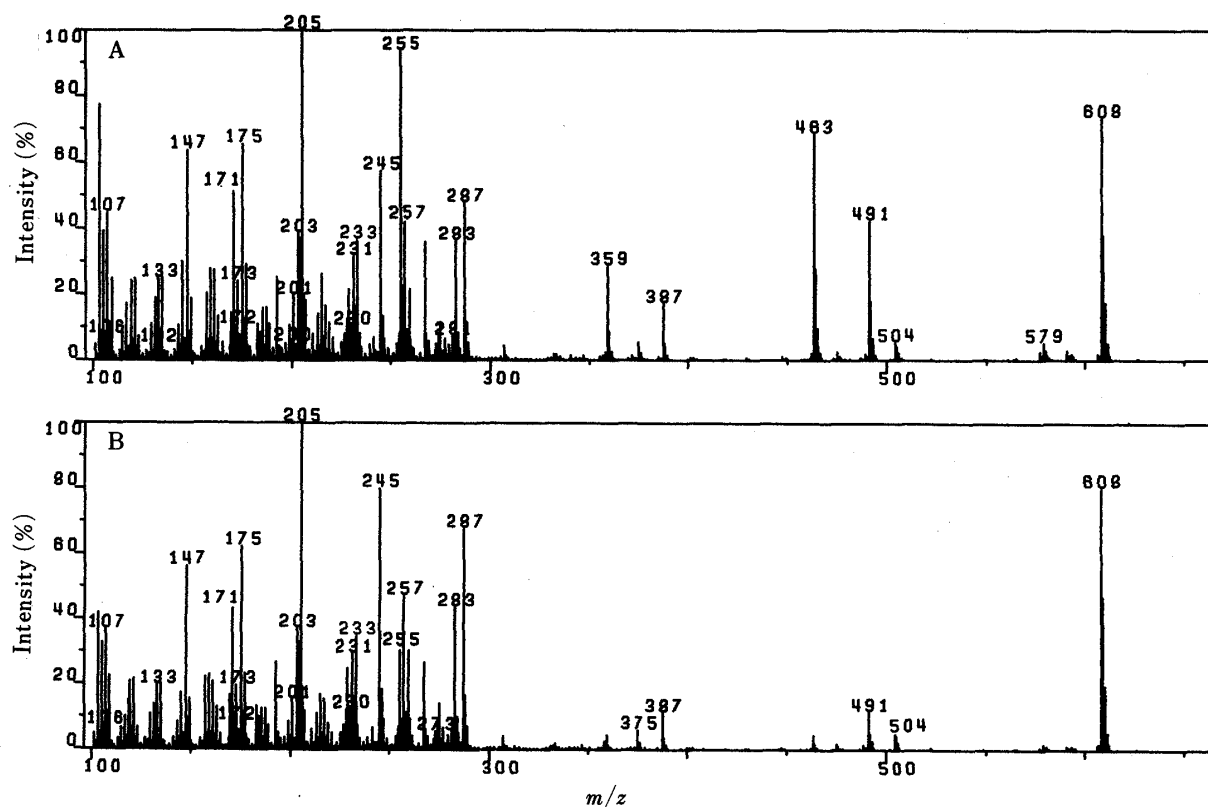


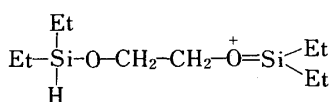
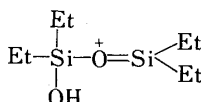
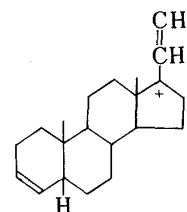
Fig. 3. Mass Spectra of the DEHS-DES Derivatives

A: 5 β -pregnane-3 α ,17 α ,20 α ,21-tetraol (**7**), B: 5 β -pregnane-3 α ,17 α ,20 β ,21-tetraol (**8**).

molecular ion, produced by simple cleavage of the C(20)–C(21) bond, afforded the ion of m/z 491, and the further elimination of DEHSOH gave the ion of m/z 387.

A major fragmentation process in this compound involves cleavage of ring D accompanied with hydrogen transfer from C-16 to C-13, whereas the TMS ethers^{12,18)} and alkyldimethylsilyl ethers^{5,6)} undergo a preferential fission at the C(17)–C(20) bond to yield the nucleic fragment with high intensity. The ion of m/z 287, characteristic of the DES group of pregnanes with a 17,20,21-triol moiety, was assigned to correspond to the ion of m/z 185 in the DEHS-DES derivatives of 17,20-diols as noted above. The appearance of ions of m/z 491 [$M - CH_2ODEHS$]⁺ and m/z 287 supported the conclusion that this DEHS-DES pregnane (**8**) is the 3,21-bis-DEHS-17,20-DES derivative.

Significant ions in the low mass region included several silicon atom-containing ions such as m/z 287, 233 and 205, and hydrocarbon fragment ions, m/z 283 and 255, whose elemental compositions were determined by GC-high resolution MS. Plausible structures of the major fragment ions, consistent with stable isotope labelling data, linked scanning spectra and accurate mass measurements, are illustrated (below). The detailed fragmentation pathways involved in the formation of these ions are now under investigation.

 m/z 233 m/z 205 m/z 283

As discussed above, the reaction product of **8** with DEHS-BSTFA was identified as the 3,21-bis-DEHS-17,20-DES derivative, whereas Brooks and Harvey¹⁵⁾ reported that alkylboronates from pregnanes with a 17,20,21-triol structure yielded the six membered boronate esters involving the 17- and 21-hydroxyl groups. The present result indicates that formation of the five membered 17,20-DES ring takes precedence over that of the 17,21-DES counterpart, and that this may be one of the characteristic features of the DES derivatives.

A great difference of mass spectral patterns between the DEHS-DES derivative of **8** and that of its 20 α -isomer (**7**) was the appearance of an intense peak at m/z 463 in the latter, as shown in Figure 3A. The relative abundance of this ion of the 20 α -isomer (**7**) was about ten times higher than that of the 20 β -isomer (**8**). However, the 3,21-bis-TBDMS-17,20-DES derivatives of **7** and **8**, both of which were prepared by treatment with TBDMS-MSTFA and then with DEHS-BSTFA, showed practically the same mass spectral pattern and gave the ion of m/z 491, corresponding to the ion of m/z 463 in the DEHS-DES derivatives, even though with low abundance.

It is not known at present whether the origin of the above remarkable difference in spectral appearance between the DEHS-DES derivatives of **7** and **8** lies in the stereoelectronic nature of the fragmentation. It might be due to the constitutional nature of the DEHS-DES compound. Thus, the structure of the reaction product of **7** with DEHS-BSTFA might correspond to one of the other two isomeric structures, **7B** or **7C** in Chart 2. Characterization of these products is now being attempted and details will be presented elsewhere. In any case, it is evident that the present DEHS-DES derivatization is very useful for the discrimination of 20 α and 20 β isomers of 17,20,21-triols.

A comparison of the spectra of the DEHS-DES derivatives of 5 β -pregnane-3 α ,11 β ,17 α ,20 α ,21-pentaol (**9**) and its 20 β -isomer (**10**) with those of **7** and **8** shows a shift of the molecular

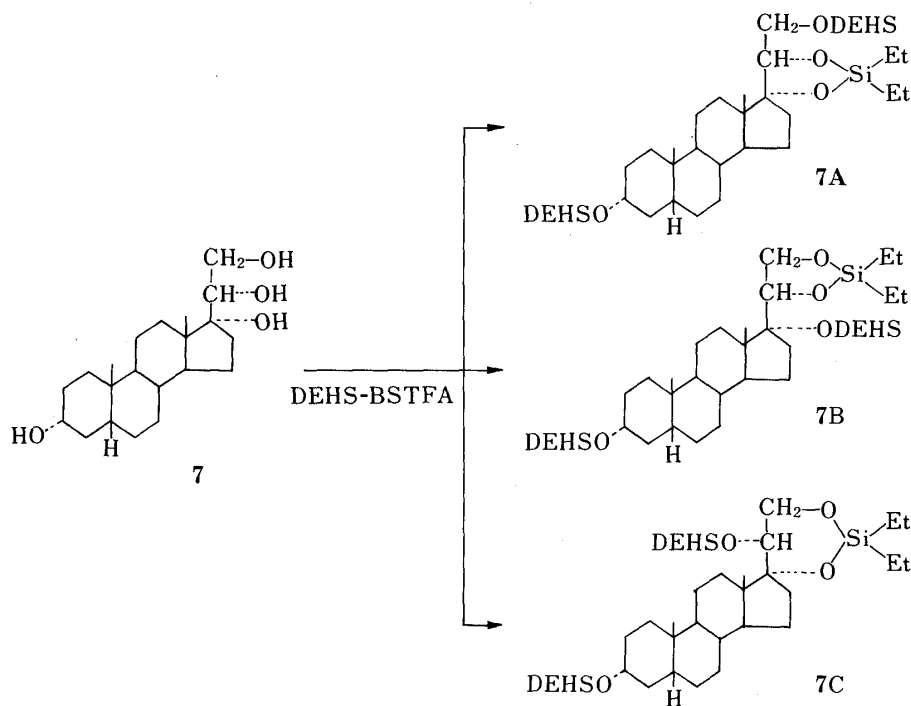


Chart 2

ion from m/z 608 to 710, indicating the incorporation of a DEHS group at the sterically hindered 11β -hydroxyl group. A similar series of ions also appeared in the spectra of 11β -hydroxylated compounds, together with additional fragment ions due to the elimination of three DEHSOH molecules from the molecular ion.

Acknowledgements The authors are grateful to Professor Dr. C. J. W. Brooks, University of Glasgow, for valuable advice and suggestion on MS fragmentations. They also thank Professor Dr. A. Tsuji, Showa University, for the generous gift of a sample of 5β -pregnane- $3\alpha,17\alpha,20\beta$ -triol.

References

- 1) R. W. Kelley, *Steroids*, **13**, 507 (1969).
- 2) G. Phillipou, D. A. Bigham, and S. F. Seemark, *Steroids*, **26**, 516 (1975).
- 3) G. Phillipou, *J. Chromatogr.*, **129**, 384 (1976).
- 4) M. A. Quilliam and J. B. Westmore, *Steroids*, **29**, 579 (1977).
- 5) H. Miyazaki, M. Ishibashi, M. Itoh, and T. Nambara, *Biomed. Mass Spectrom.*, **4**, 23 (1977).
- 6) H. Miyazaki, M. Ishibashi, and K. Yamashita, *Biomed. Mass Spectrom.*, **6**, 57 (1979).
- 7) D. J. Harvey, *Biomed. Mass Spectrom.*, **7**, 211 (1980).
- 8) D. J. Harvey, *Biomed. Mass Spectrom.*, **7**, 278 (1980).
- 9) M. A. Quilliam and J. B. Westmore, *Eur. J. Mass Spectrom. Biochem. Med. Environ. Res.*, **1**, 53 (1980).
- 10) S. G. Andersson and J. Sjövall, *J. Chromatogr.*, **289**, 195 (1984).
- 11) G. M. Anthony, C. J. W. Brooks, I. Maclean, and I. Sangster, *J. Chromatogr. Sci.*, **7**, 623 (1969).
- 12) T. A. Baillie, C. J. W. Brooks, and B. S. Middleditch, *Anal. Chem.*, **44**, 30 (1972).
- 13) H. Miyazaki, M. Ishibashi, M. Itoh, and K. Yamashita, *Biomed. Mass Spectrom.*, **11**, 377 (1984).
- 14) P. M. J. Van den Berg and T. P. Cox, *Chromatographia*, **5**, 301 (1971).
- 15) C. J. W. Brooks and D. J. Harvey, *J. Chromatogr.*, **54**, 193 (1971).
- 16) C. J. W. Brooks, B. S. Middleditch, and D. J. Harvey, *Org. Mass Spectrom.*, **5**, 1429 (1971).
- 17) P. Vouros and D. J. Harvey, *J. Chem. Soc. Perkin Trans. 1*, **1973**, 727.
- 18) P. Vouros and D. J. Harvey, *Biomed. Mass Spectrom.*, **7**, 217 (1980).