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Blockage of Coordination with Shift Reagent by *tert*-Butyldimethylsilylation in Nuclear Magnetic Resonance Spectroscopy and Its Applications to Deuterium-Labeled Steroids[1]

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Derivatization of a hydroxyl group into the *tert*-butyldimethylsilyl ether was shown to be useful for selective blockage of coordination of the substrate with the lanthanide shift reagent in ^{1}H nuclear magnetic resonance spectroscopy. This method was conveniently applied to confirmation of the labeled position with deuterium in the dioxygenated steroids.

Keywords——^{1}H NMR spectroscopy; lanthanide shift reagent; hydroxylated steroids; *tert*-butyldimethylsilylation; blockage of coordination; deuterium labeling

The deuterium labeled steroids are widely used as substrate for the studies on the steric mechanism of biotransformation. The location and configuration of a heavy isotope labeled in a molecule is determined by a variety of physical methods. However, these techniques are not always satisfactory due to the inevitable limitations. The use of the lanthanide shift reagent in ^{1}H nuclear magnetic resonance (NMR) spectroscopy appears to be promising for solving the problem because of its high utility for separation of the overlapping signals under the methylene-methine envelope. This technique, however, is not necessarily applicable to the commonly occurring dioxygenated steroids. Difficulties are encountered with the interpretation of the spectra, since both hydroxyl and oxo groups coordinate with the shift reagent and the equilibrium constants for the complex formation at the two sites are dependent upon the steric factors. In addition, these substrates are usually sparingly soluble in deuteriochloroform and carbon tetrachloride, which are widely used for the shift reagent as solvent. Accordingly, it is desirable to convert one of the functional groups into the appropriate derivative which is incapable of coordinating with the shift reagent and besides brings about the improved solubility of the substrate in the non-polar solvent.

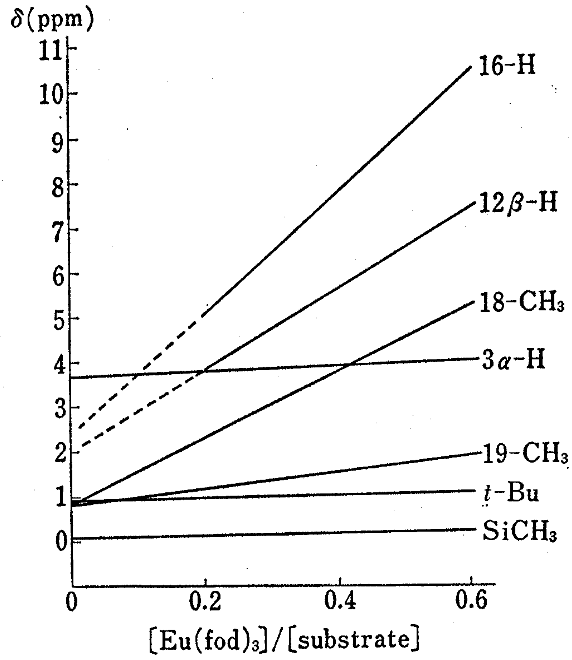

Fig. 1. Chemical Shifts of Protons of Isoandrosterone *tert*-Butyldimethylsilyl Ether (1) induced by Coordination with Eu(fod)$_3$

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[1] Part CXXI of "Studies on Steroids" by T. Nambara; Part CXX: T. Nambara and Y. Kawarada, *Chem. Pharm. Bull.* (Tokyo), 25, 942 (1977). A part of this work has been presented as a preliminary communication, H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Ind.* (London), 1975, 650.

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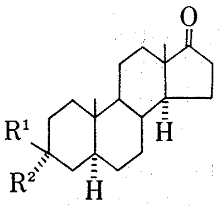
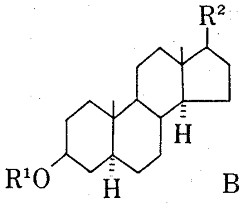
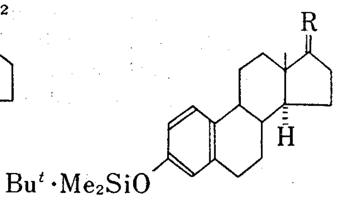
Derivatization of a hydroxyl function into the alkyl ether may be one of the methods, since coordination of the ether with the shift reagent is relatively weak.[3] Nevertheless, methyl or methylene protons at the α-position of the ethereal linkage resonate at the lower magnetic field, may sometimes cause the spectrum to be complicated.[4] Furthermore, contributions of the ether and free oxo groups to coordination with the shift reagent are not always distinctly separated. Although several methods such as trifluoroacetylation,[5] tosylation,[6,7] and trimethylsilylation[7] have been proposed, the use of a more appropriate derivative which is stable under a variety of reaction conditions, is requisite. From these points of view the *tert*-butyldimethylsilyl ether seems to be suitable. The present paper deals with the ^{1}H NMR spectral studies on the steroid *tert*-butyldimethylsilyl ethers with the shift reagents, tris(dipivalomethanato)europium (III) [Eu(dpm)$_3$] and tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium (III) [Eu(fod)$_3$], in carbon tetrachloride. Silylation was carried out according to Corey's procedure[8] with or without a slight modification. The former



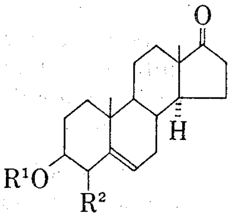
1 : $R^1=OSiMe_2\cdot Bu^t$, $R^2=H$
2 : $R^1=H$, $R^2=OSiMe_2\cdot Bu^t$



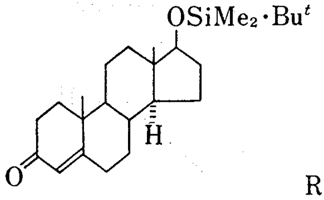
3 : $R^1=SiMe_2\cdot Bu^t$, $R^2=OH$
4 : $R^1=H$, $R^2=OSiMe_2\cdot Bu^t$
8 : $R^1=SiMe_2\cdot Bu^t$, $R^2=COCH_3$



5 : R=O
6 : $R=\langle^{OH}_{H}$



7 : $R^1=SiMe_2\cdot Bu^t$, $R^2=H$
21 : $R^1=H$, $R^2=D$



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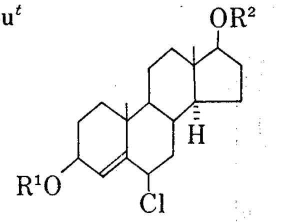
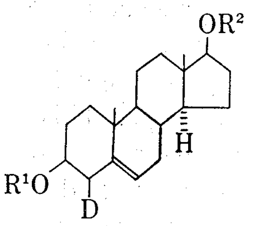
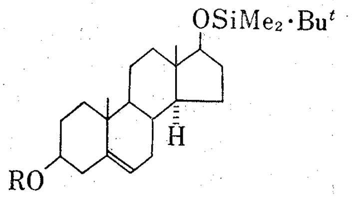


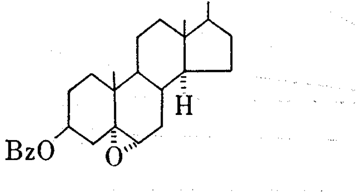
11 : $R^1=R^2=Bz$
20 : $R^1=Bz$, $R^2=SiMe_2\cdot Bu^t$



12 : $R^1=R^2=H$
13 : $R^1=R^2=SiMe_2\cdot Bu^t$
15 : $R^1=H$, $R^2=SiMe_2\cdot Bu^t$



14 : R=H
16 : R=Ac
17 : R=Bz



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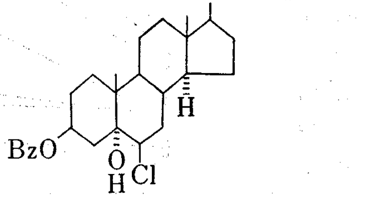

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Chart 1

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3) J.K.M. Sanders and D.H. Williams, *J. Am. Chem. Soc.*, **93**, 641 (1971).
4) J.E. Herz, V.M. Rodriquez, and P. Joseph-Nathan, *Tetrahedron Lett.*, **1971**, 2949.
5) D.R. Crump, J.K.M. Sanders, and D.H. Williams, *Tetrahedron Lett.*, **1970**, 4949; A. Ius, G. Vecchio, and G. Carrea, *ibid.*, **1972**, 1543.
6) G. Lukacs, X. Lusinchi, P. Girard, and H. Kagan, *Bull. Soc. Chim. Fr.*, **1971**, 3200.
7) P. Girard, H. Kagan, and S. David, *Tetrahedron*, **27**, 5911 (1971).
8) E.J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972).

method employing pyridine–dimethylformamide (1:2) as a solvent was profitable, since the reaction proceeded with relative ease.

An initial study was directed to the NMR spectral change of isoandrosterone *tert*-butyldimethylsilyl ether (**1**) in the presence of various concentrations of Eu(fod)$_3$ at 23°. As illustrated in Fig. 1, a plot of induced shift value *vs.* the molar ratio of Eu(fod)$_3$ to the substrate showed a good linearity, respectively. The chemical shifts of *tert*-butyldimethylsilyl and C-3 protons are not so remarkably affected as those of C-16 and 18-methyl protons, which are located close to the 17-oxo group. The similar phenomenon was also observed when androsterone silyl ether (**2**), that is the C-3 epimer, was employed as a substrate. This finding implies that there is no substantial difference in the mode of blockage of coordination with the shift reagent between the epimeric silyl ethers. Considering the absence of any significant hindrance at C-3 in isoandrosterone, the selective blockage of coordination with the shift reagent by *tert*-butyldimethylsilylation appeared to be effective for other hydroxysteroids. This was verified with the silyl ethers of representative hydroxysteroids including 3β-hydroxy-5α-pregnan-20-one (**8**) and testosterone (**10**).

Measurements of the NMR spectra of 5α-androstane-3β,17β-diol 3- and 17-*tert*-butyldimethylsilyl ethers (**3**,**4**) using Eu(dpm)$_3$ as a shift reagent were then undertaken. At the value of 0.4—0.5 in the molar ratio of the shift reagent to the silyl ether the signals of 17α, 16β, 16α and 18-methyl protons in **3** and of 3α, 2β, 2α, 4β and 4α protons in **4** were shifted to the lower field, while the induced shift values of protons near the silyloxy group were small and substantially negligible (Table I). Transformation of the dioxygenated compound into the *tert*-butyldimethylsilyl ether proved bo be useful for selective blockage of coordination with these shift reagents.

The present method was further applied to confirmation of the deuterium labeled position. In a previous paper of this series the preparation of deuterioestrones labeled at C-14, C-15 and C-16 has been reported.[9] The deuterated substrates were readily converted to the

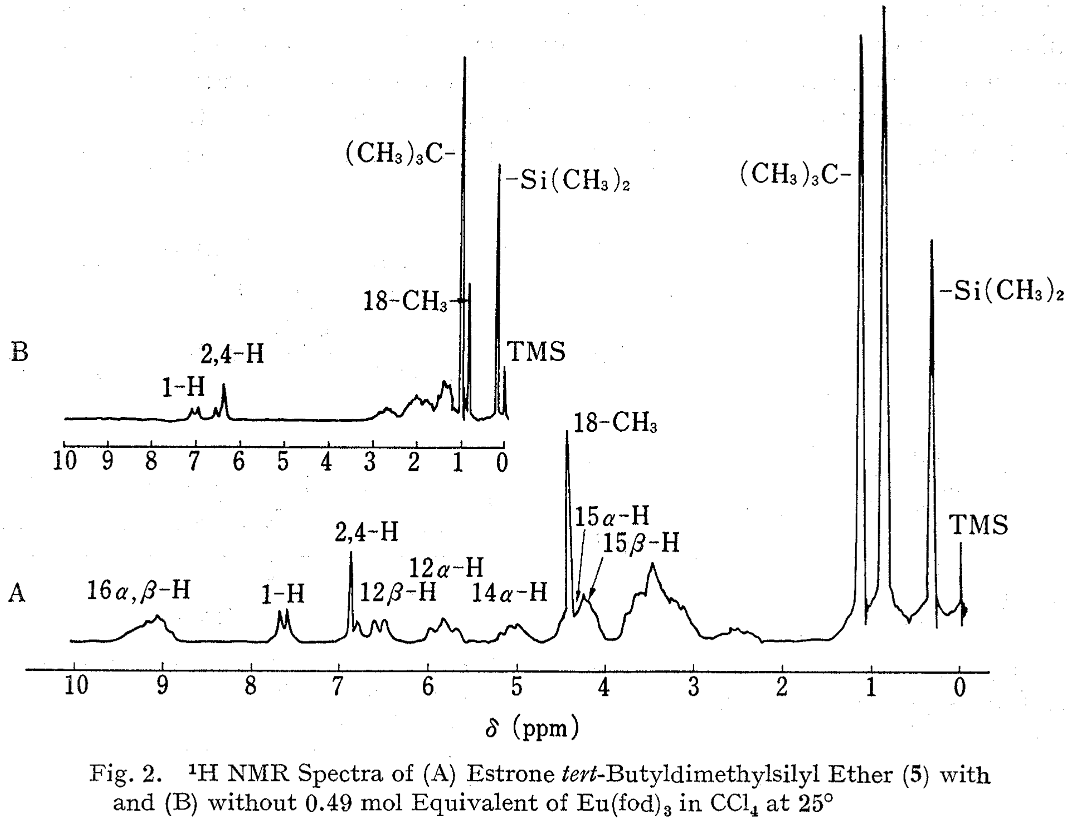

Fig. 2. ^{1}H NMR Spectra of (A) Estrone *tert*-Butyldimethylsilyl Ether (**5**) with and (B) without 0.49 mol Equivalent of Eu(fod)$_3$ in CCl$_4$ at 25°

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[9] H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **24**, 380 (1976).

tert-butyldimethylsilyl ethers by treatment with *tert*-butyldimethylsilyl chloride in the presence of imidazole in dimethylformamide. The NMR spectra of non-labeled estrone silyl ether (**5**) with or without the shift reagent are illustrated in Fig. 2A and 2B, respectively. Without the use of the shift reagent only the signals of aromatic, 18-methyl and *tert*-butyldimethylsilyl protons were assignable. Upon the addition of Eu(fod)$_3$, however, a remarkable change in the NMR spectrum did take place permitting us to assign the signals with more ease. As for the deuterated estrone 3-silyl ethers the NMR signals due to 14α, 15α, 15β, 16α and 16β protons disappeared, respectively. The unchanged signals at 5.8 and 6.6 ppm were assignable to 12α and 12β protons as judged from their splitting patterns. As shown in Fig. 2A, the signals of 12α, 12β and 14α protons were well resolved, while those of epimeric protons at C-15 and C-16 were not discriminated one another even when a larger amount of the shift reagent was added.

The spectral analysis under the similar conditions was then carried out with the 17β-hydroxyl compound. The NMR spectrum of estradiol 3-*tert*-butyldimethylsilyl ether (**6**) and Eu(fod)$_3$ in a molar ratio of 2 to 1 is illustrated in Fig. 3. The signals of epimeric protons were readily distinguished as predicted by the McConell-Robertson expression. The orientation of deuterium introduced in the steroid nucleus could be unambiguously assigned by inspection of the spectra of the labeled substrates, derivable from deuterioestrone silyl ethers. The results obtained justified the mechanism of stereochemical reactions employed for the preparation of the deuterated compounds, *i.e.* reductive cleavage of the epoxide and displacement of the sulfate with lithium aluminum deuteride, and deuterioboration of the double bond. In addition, the present study offered an unequivocal assignment of the signals of ring D and C-12 protons.

In connection with the steric mechanism of biotransformations such as isomerization to the Δ^4-3-ketone and aromatization to estrogen, the metabolic fate of C-4 protons in dehydroepiandrosterone is of considerable interest. Hence, stereospecific labeling with deuterium at this position is required. The S_N2' displacement of 6β-chlorine in the 3β-benzoyloxy-Δ^4-6β-chloro derivative with lithium aluminum deuteride has been used for the 4β-labeling.

The stereospecificity of the labeled isotope has been demonstrated by the chemical reactions and the infrared spectroscopy.[10,11] However, it is desirable to be able to confirm the stereochemistry and isotopic purity of the label with relative ease. The present technique

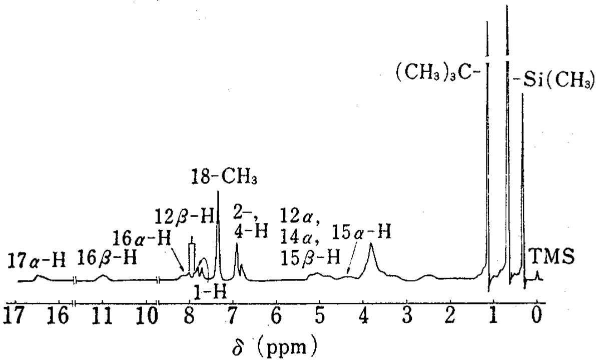

Fig. 3. ^{1}H NMR Spectrum of Estradiol 3-*tert*-Butyldimethylsilyl Ether (**6**) with 0.55 mol Equivalent of Eu(fod)$_3$ in CCl$_4$ at 23°

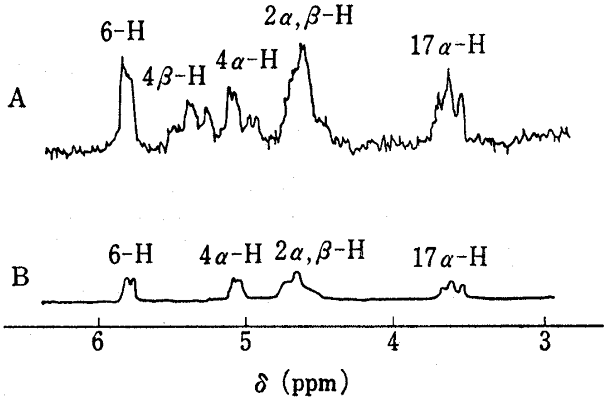

Fig. 4. ^{1}H NMR Spectra of (A) 5-Androstene-3β,17β-diol *tert*-Butyldimethylsilyl Ether (**14**) and (B) Its 4β-Deuterio Derivative (**15**) with 0.20 mol Equivalent each of Eu(fod)$_3$ in CCl$_4$ at 24°

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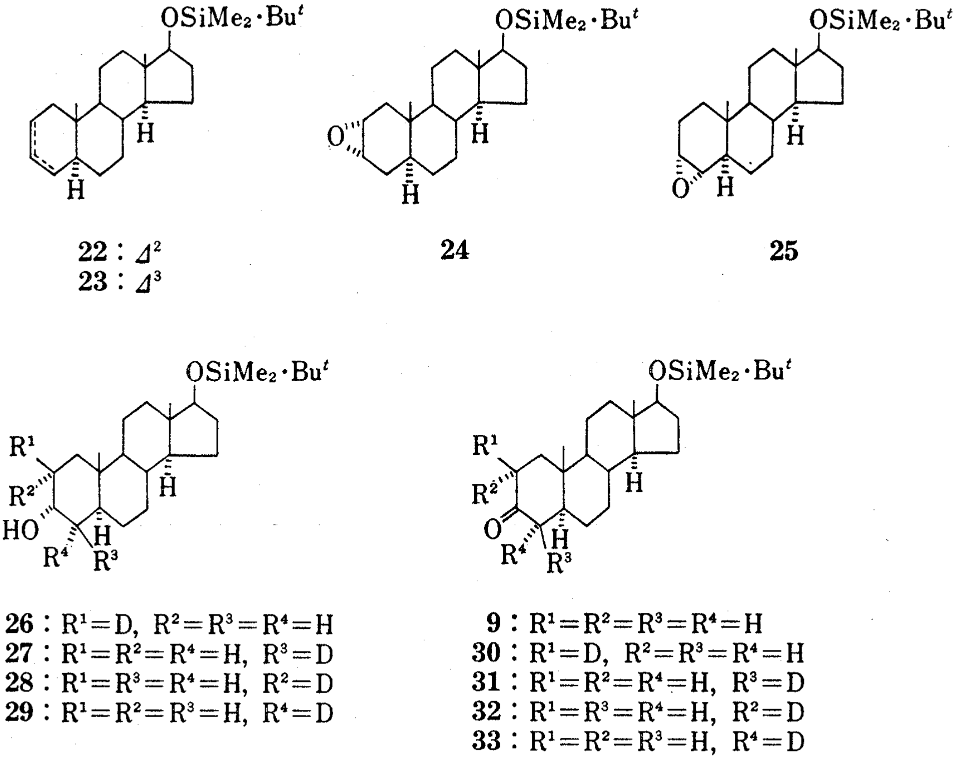
[10] R.E. Ireland, T.I. Wrigley, and W.G. Young, *J. Am. Chem. Soc.*, **81**, 2818 (1959).

[11] S.K. Malhotra and H.J. Ringold, *J. Am. Chem. Soc.*, **87**, 3228 (1965).

proved to be more convenient for this purpose. The monodeuterated 5-androstene-3β,17β-diol (**12**) obtained from 6β-chloroandrost-4-ene-3β,17β-diol dibenzoate (**11**) by treatment with lithium aluminum deuteride in anhydrous ether was converted to the disilyl ether (**13**). Partial hydrolysis of **13** with 5 N hydrochloric acid in acetone provided the 17-monosilyl ether (**15**) in 70% yield. Stereospecific introduction of deuterium at the 4β position was verified by comparison of the NMR spectrum of **15** (Fig. 4B) with that of the non-labeled substrate (**14**) (Fig. 4A) at similar concentrations of $Eu(fod)_3$. As illustrated in Fig. 4B, the signal of 4β-proton disappeared almost entirely and that of 4α-proton changed from broad quartet to narrow multiplet. Besides the reliable assignment of the labeled position the present procedure is of advantage in feasibility to transform **14** into dehydroepiandrosterone. Compound **15** was also obtained from the more appropriate intermediate (**20**), where the 17β-hydroxyl group was protected as a silyl ether. Oxidation of 3β-benzoyloxy-5-androsten-17β-ol *tert*-butyldimethyl silyl ether (**17**) with *m*-chloroperbenzoic acid afforded the 5α,6α-epoxide (**18**), which on treatment with hydrochloric acid was transformed into the chlorohydrin (**19**). Being dehydrated with thionyl chloride in pyridine, **19** was led to the desired 3β-benzoyloxy-Δ^4-6β-chloro derivative (**20**). The S_N2' displacement of **20** in the manner as with **11** furnished **15** in a satisfactory yield. Subsequent acetylation, desilylation, oxidation and saponification can provide 4β-deuteriodehydroepiandrosterone (**21**).



22 : Δ^2
23 : Δ^3

24

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26 : $R^1=D$, $R^2=R^3=R^4=H$
27 : $R^1=R^2=R^4=H$, $R^3=D$
28 : $R^1=R^3=R^4=H$, $R^2=D$
29 : $R^1=R^2=R^3=H$, $R^4=D$

9 : $R^1=R^2=R^3=R^4=H$
30 : $R^1=D$, $R^2=R^3=R^4=H$
31 : $R^1=R^2=R^4=H$, $R^3=D$
32 : $R^1=R^3=R^4=H$, $R^2=D$
33 : $R^1=R^2=R^3=H$, $R^4=D$

Chart 2

In Table I the NMR spectral data of the steroid *tert*-butyldimethylsilyl ethers using the shift reagents are collected. The signals of the C-18, C-19, *tert*-butyl, silyl-methyl and characteristic protons could easily be assigned. The assignment of the signals due to other protons in ring A, C and D was based on the spin-coupling sequence and comparison with the data of the analogs and deuterium labeled derivatives. The deuteration experiment with C_{18} steroids permitted us to assign the signals of ring C and D protons in **1**, **2**, **3** and **7**. The shifting pattern of ring A protons in **4** was quite similar to that reported for the analogs lacking the oxygen

Table I. Chemical Shifts and Signal Patterns in the Presence of Shift Reagents[a]

Compound	Shift reagent[b]	E/S[c]	Temp.	$H_{16}\beta$	$H_{16}\alpha$	$H_{12}\beta$	$H_{12}\alpha$	$H_{14}\alpha$	$H_{15}\alpha$	$H_{15}\beta$
1	A	0.44	24°	(7.90—8.30) m		5.80bd	5.00bt	4.32m	(3.6—4.0) m	
2	A	0.59	31°	(7.75—8.05) m		5.72bd	5.05bt	4.38m	(3.5—3.9) m	
7	A	0.68	24°	(11.00—11.80) m		8.20bd	7.19bt	6.20m	5.25m	5.00m
3	B	0.46	22°	8.76m	6.55m	6.08bd	(3.5—4.4) m			
8	A	0.51	25°	8.94m[d]	5.1m[d]	5.24bd	u	u	u	u

Compound	C_{18}-CH_3	C_{19}-CH_3	*t*-Bu	Si-CH_3	$H_3\alpha$	$H_3\beta$	$H_{17}\alpha$	Δ^5-H	C_{21}-CH_3
1	3.92 s	1.51 s	0.99 s	0.12 s	3.8m				
2	3.73 s	1.40 s	0.96 s	0.12 s		4.16m			
7	5.80 s	2.14 s	1.08 s	0.14 s	u			6.08m	
3	5.72 s	1.61 s	1.00 s	0.16 s	u		13.96m		
8	4.41 s	1.28 s	1.00 s	0.17 s	3.8m		7.79 t		9.25 s

Compound	Shift reagent[b]	E/S[c]	Temp.	$H_2\alpha$	$H_2\beta$	$H_4\beta$	$H_4\alpha$	$H_1\alpha$	$H_1\beta$	$H_5\alpha$
4	B	0.36	22°	(6.65—7.40) m			6.48m	(2.7—3.45) m		
14	A	0.20	24°	(4.30—4.80) m		5.40bt	5.04dd	u	u	
9	A	0.60	24°	9.50bd	8.60m	8.48 t	9.32bd	4.44bt	4.02dd	4.80m
10	A	0.53	33°	9.70bd	9.40bt			4.71sx	4.32dd	

Compound	C_{19}-CH_3	C_{18}-CH_3	*t*-Bu	Si-CH_3	$H_{17}\alpha$	$H_3\alpha$	Δ^4-H	Δ^5-H
4	2.50 s	1.00 s	0.95 s	0.11 s 0.09 s	3.68 t	11.70m		
14	1.92 s	0.88 s	0.91 s	0.02 s	3.64 t	7.90m		5.82m
9	2.83 s	1.12 s	1.00 s	0.13 s 0.11 s	3.80 t			
10	2.81 s	1.16 s	1.01 s	0.15 s 0.12 s	3.76 t		13.64 s	

a) u, unresolved; s, singlet; d, doublet; t, triplet; sx, sextet; m, multiplet; b, broad.
b) A, Eu(fod)$_3$; B, Eu(dpm)$_3$.
c) E/S, molar ratio of shift reagent to steroid.
d) Tentatively assigned.

function at C-17.[12] With regard to **9** the signal assignment was confirmed by the use of the deuterated substrates (**30**, **31**, **32**, **33**). These were prepared by deuterioboration of the olefines (**22**, **23**) and reductive cleavage of the epoxides (**24**, **25**) with lithium aluminum deuteride, followed by oxidation of the hydroxyl derivatives (**26**, **27**, **28**, **29**) with chromic anhydride-pyridine complex under the protection of the 17β-hydroxyl group as a silyl ether.

It has been demonstrated that derivatization of a hydroxyl group into the *tert*-butyldimethylsilyl ether is of great use for selective blockage of coordination of the substrate with the lanthanide shift reagent in NMR spectroscopy. This method is profitable in that the silyl ether is stable during the deuterium labeling reactions with lithium aluminum deuteride, sodium borodeuteride or deuteriodiborane, and also in that unless the resolution of the spectrum can be satisfactorily attained with the keto substrate, one can convert it to the cor-

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[12] O.R. Rodig and P.P. Roller, *Org. Magnetic Resonance*, **6**, 264 (1974).

responding hydroxyl derivative, and *vice versa*. Considering the frequent use of the silylated derivative in gas chromatography-mass spectrometry[13] and demand of the appropriately deuterated compound as an internal standard, the present method is much more convenient than the hitherto known. It may be widely applicable to the structural elucidation of the synthetic and naturally occurring substances as well as steroids and also to ^{13}C NMR spectroscopy because of the relative ease in silylation and desilylation under the mild conditions.

The studies on the steric mechanism of microbial transformation of steroids employing the present technique are being conducted in these laboratories, and the details will be reported in the near future.

Experimental

Measurement of NMR Spectra

NMR spectra were recorded on a JEOL Model PS-100 spectrometer at 100 MHz with *ca.* 0.08 M substrate in CCl_4. Chemical shifts are quoted as ppm (δ) downfield from tetramethylsilane used as an internal standard. The shift reagents were stored *in vacuo* over P_2O_5 until the use.

Synthesis of Samples[14]

Isoandrosterone *tert*-butyldimethylsilyl ether (**1**), androsterone *tert*-butyldimethylsilyl ether (**2**), 5α-androstane-3β,17β-diol 17-*tert*-butyldimethylsilyl ether (**4**), estrone *tert*-butyldimethylsilyl ether (**5**), estradiol 3-*tert*-butyldimethylsilyl ether (**6**), dehydroepiandrosterone *tert*-butyldimethylsilyl ether (**7**) and testosterone *tert*-butyldimethylsilyl ether (**10**) were prepared by the methods described in the previous papers.[9,15]

General Procedure for Preparation of *tert*-Butyldimethylsilyl Ether——To a solution of steroid (0.1 mmol) in dimethylformamide (2 ml) or pyridine (0.5 ml)–dimethylformamide (1 ml) were added *tert*-butyldimethylsilyl chloride (0.5—2 mmol) and imidazole (1—4 mmol), and the solution was allowed to stand at room temperature for 2 hr. The reaction mixture was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After removal of the solvent and excess reagent by evaporation under reduced pressure the crude product obtained was purified, if necessary, by preparative TLC and recrystallization from the appropriate solvent.

3β-Hydroxy-5α-pregnan-20-one *tert*-Butyldimethylsilyl Ether (8)——Colorless needles (from MeOH), mp 142—143°, $[\alpha]_D$ +75.0° (c=0.1). *Anal.* Calcd. for $C_{27}H_{48}O_2Si$: C, 74.94; H, 11.18. Found: C, 74.88; H, 11.14.

5α-Dihydrotestosterone *tert*-Butyldimethylsilyl Ether (9)——Colorless needles (from MeOH), mp 134—134.5°, $[\alpha]_D$ +27.3° (c=0.1). *Anal.* Calcd. for $C_{25}H_{14}O_2Si$: C, 74.20; H, 10.96. Found: C, 73.97; H, 10.81.

3β-Acetoxy-5-androsten-17β-ol *tert*-Butyldimethylsilyl Ether (16)——Colorless needles (from MeOH), mp 133.5—134°, $[\alpha]_D$ −54.0° (c=0.3). *Anal.* Calcd. for $C_{27}H_{46}O_3Si$: C, 72.59; H, 10.38. Found: C, 72.43; H, 10.50.

3β-Benzoyloxy-5-androsten-17β-ol *tert*-Butyldimethylsilyl Ether (17)——Colorless plates (from MeOH), mp 131—133°, $[\alpha]_D$ −18.0° (c=0.2). *Anal.* Calcd. for $C_{32}H_{48}O_3Si$: C, 75.54; H, 9.51. Found: C, 75.80; H, 9.78.

5α-Androst-2-en-17β-ol *tert*-Butyldimethylsilyl Ether (22)——Colorless plates (from acetone), mp 94—95°, $[\alpha]_D$ +45.8° (c=0.1). *Anal.* Calcd. for $C_{25}H_{44}OSi$: C, 77.25; H, 11.41. Found: C, 77.22; H, 11.16.

5α-Androst-3-en-17β-ol *tert*-Butyldimethylsilyl Ether (23)——Colorless needles (from acetone), mp 91—92°, $[\alpha]_D$ +59.1° (c=0.1). *Anal.* Calcd. for $C_{25}H_{44}OSi$: C, 77.25; H, 11.41. Found: C, 77.08; H, 11.35.

2α,3α-Epoxy-5α-androstan-17β-ol *tert*-Butyldimethylsilyl Ether (24)——Colorless plates (from acetone), mp 120—121°, $[\alpha]_D$ +35.0° (c=0.1). *Anal.* Calcd. for $C_{25}H_{44}O_2Si$: C, 74.20; H, 10.96. Found: C, 74.19; H, 10.84.

3α,4α-Epoxy-5α-androstan-17β-ol *tert*-Butyldimethylsilyl Ether (25)——Colorless plates (from MeOH), mp 149—149.5°, $[\alpha]_D$ +30.3° (c=0.3). *Anal.* Calcd. for $C_{25}H_{44}O_2Si$: C, 74.20; H, 10.96. Found: C, 74.10; H, 11.14.

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13) G. Phillipou, D.A. Bigham, and R.F. Seamark, *Steroids*, **26**, 516 (1975); S.J. Gaskell and C.J.W. Brooks, *Biochemical Society Transactions*, **4**, 111 (1976); R.W. Kelly and P.L. Taylor, *Anal. Chem.*, **48**, 465 (1976).

14) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured at 20—25° in $CHCl_3$. For preparative thin-layer chromatography (TLC) silica gel H (E. Merck AG, Darmstadt) was used as an adsorbent. Isotopic purity of lithium aluminum deuteride used was over 99%. Mixed melting point of the deuterated compound with the non-labeled authentic sample showed no depression, respectively.

15) H. Hosoda, D.K. Fukushima, and J. Fishman, *J. Org. Chem.*, **38**, 4209 (1973); H. Hosoda, K. Yamashita, H. Sagae, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **23**, 2118 (1975).

5α-Androstane-3β,17β-diol 3-*tert*-Butyldimethylsilyl Ether (3)——To a solution of **1** (100 mg) in tetrahydrofuran (3 ml)–H_2O (1 ml) was added $NaBH_4$ (100 mg) at 0° and allowed to stand at room temperature for 1 hr. After addition of 10% AcOH (3 ml) to decompose the excess reagent the solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from aq. MeOH gave **3** (92 mg) as colorless leaflets, mp 177—178°, $[\alpha]_D$ +6.7° (c=0.6). *Anal.* Calcd. for $C_{25}H_{46}O_2Si$: C, 73.83; H, 11.40. Found: C, 73.60; H, 11.57.

5-Androstene-3β,17β-diol 17-*tert*-Butyldimethylsilyl Ether (14)——**16** (100 mg) was dissolved in 5% methanolic KOH (8 ml) and allowed to stand at room temperature for 1 hr. The reaction mixture was diluted with ethyl acetate, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from MeOH gave **14** (70 mg) as colorless leaflets, mp 142—143°, $[\alpha]_D$ −40.0° (c=0.2). *Anal.* Calcd. for $C_{25}H_{44}O_2Si$: C, 74.20; H, 10.96. Found: C, 73.75; H, 10.92.

4β-Deuterio-5-androstene-3β,17β-diol 17-*tert*-Butyldimethylsilyl Ether (15)——i) From **11**: To a solution of 6β-chloro-4-androstene-3β,17β-diol dibenzoate (11)[11] (120 mg) in anhydrous ether was added $LiAlD_4$ (50 mg) and refluxed for 10 hr. After addition of moist ethyl acetate to decompose the excess reagent the resulting solution was diluted with 25% Rochelle salt solution and extracted with ethyl acetate. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the 3β,17β-diol (**12**) (40 mg) as a crystalline material. To a solution of **12** in dimethylformamide (2 ml) were added *tert*-butyldimethylsilyl chloride (200 mg) and imidazole (400 mg), and the solution was allowed to stand at room temperature for 12 hr. The reaction mixture was diluted with ether, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by preparative TLC using hexane–ethyl acetate (10: 1) as developing solvent to give the 3,17-disilyl ether (**13**) (50 mg) as a crystalline material. To a solution of **13** in acetone (6 ml) was added 5N HCl (0.2 ml) and allowed to stand at room temperature for 15 min. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by preparative TLC using hexane–ethyl acetate (5: 1) as developing solvent. Recrystallization from MeOH gave **15** (35 mg) as colorless leaflets, mp 142—143°.

ii) From 3β-Benzoyloxy-6β-chloro-4-androsten-17β-ol *tert*-Butyldimethylsilyl Ether (20): **20** (60 mg) was treated with $LiAlD_4$ in the manner as described with **11**. Purification of the eluate from MeOH gave **15** (20 mg) as colorless leaflets, mp 142—143.°

3β-Benzoyloxy-5α,6α-epoxyandrostan-17β-ol *tert*-Butyldimethylsilyl Ether (18)——To a solution of **17** (626 mg) in benzene was added *m*-chloroperbenzoic acid (600 mg) and allowed to stand at room temperature for 12 hr. The resulting solution was diluted with ether, washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O successively, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from acetone gave **18** (227 mg) as colorless leaflets, mp 191—193°, $[\alpha]_D$ −20.0° (c=0.2). *Anal.* Calcd. for $C_{32}H_{48}O_4Si$: C, 73.24; H, 9.22. Found: C, 73.35; H, 9.49.

3β-Benzoyloxy-6β-chloroandrostane-5α,17β-diol 17-*tert*-Butyldimethylsilyl Ether (19)——To a solution of **18** (162 mg) in acetone was added conc. HCl (0.1 ml) and allowed to stand at room temperature for 30 min. The resulting solution was diluted with ethyl acetate, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Since desilylation took place to a certain extent, the residue was resilylated with *tert*-butyldimethylsilyl chloride (200 mg) and imidazole (500 mg) in pyridine (1 ml)–dimethylformamide (2 ml). After usual work-up the crude product obtained was chromatographed on silica gel (20 g). Elution with hexane–ethyl acetate (15: 1) and recrystallization of the eluate from MeOH gave **19** (125 mg) as colorless leaflets, mp 222—225°, $[\alpha]_D$ −25.0° (c=0.2). *Anal.* Calcd. for $C_{32}H_{49}ClO_4Si$: C, 68.48; H, 8.80. Found: C, 68.85; H, 9.20.

3β-Benzoyloxy-6β-chloro-4-androsten-17β-ol *tert*-Butyldimethylsilyl Ether (20)——To a solution of **19** (95 mg) in pyridine (2 ml) was added thionyl chloride (0.1 ml) and allowed to stand at 0° for 20 min. After addition of moist ether to decompose the excess reagent the resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from ether–MeOH gave **20** (50 mg) as colorless plates, mp 173.5—174°, $[\alpha]_D$ −78.0° (c=0.2). *Anal.* Calcd. for $C_{32}H_{47}O_3ClSi$: C, 70.75; H, 8.72. Found: C, 71.14; H, 8.95.

2β-Deuterio-5α-androstane-3α,17β-diol 17-*tert*-Butyldimethylsilyl Ether (26)——To a solution of **24** (60 mg) in anhydrous tetrahydrofuran (6 ml) was added $LiAlD_4$ (50 mg) and refluxed for 4 hr. After addition of moist ethyl acetate to decompose the excess reagent the resulting solution was diluted with 25% Rochelle salt and extracted with ethyl acetate. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–ethyl acetate (4: 1) as developing solvent. Recrystallization of the eluate from acetone gave **26** (55 mg) as colorless plates, mp 160.5—162°. The non-labeled sample: $[\alpha]_D$ +15.5° (c=0.2). *Anal.* Calcd. for $C_{25}H_{46}O_2Si$: C, 73.83; H, 11.40. Found: C, 73.67; H, 11.19.

4β-Deuterio-5α-androstane-3α,17β-diol 17-*tert*-Butyldimethylsilyl Ether (27)——Reduction of **25** (30 mg) with $LiAlD_4$ (30 mg) was carried out in the manner as described with **24**. Recrystallization of the crude product from acetone gave **27** (20 mg) as colorless plates, mp 159—160°.

2α-Deuterio-5α-androstane-3α,17β-diol 17-*tert*-Butyldimethylsilyl Ether (28)——To a stirred solution of **22** (100 mg) and $LiAlD_4$ (150 mg) in anhydrous ether (20 ml) was added boron trifluoride-etherate (2.5 g) in anhydrous ether (10 ml) dropwise at 0° over a period of 30 min under N_2 gas stream. The ice-bath was then removed and the reaction mixture was stirred at room temperature for 1 hr. After addition of moist ether to decompose the excess reagent the resulting solution was extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. To the residue dissolved in tetrahydrofuran (10 ml) were added 30% H_2O_2 (10 ml) and 10% NaOH (5 ml) and stirred at 0° for 1 hr. The resulting solution was diluted with ether, washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O successively, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–ethyl acetate (4: 1) as developing solvent. Recrystallization of the eluate from acetone gave **28** (50 mg) as colorless plates, mp 158—159°.

4α-Deuterio-5α-androstane-3α,17β-diol 17-*tert*-Butyldimethylsilyl Ether (29)——Treatment of **23** (100 mg) with deuteriodiborane and subsequent oxidation of the organoborane with H_2O_2–NaOH were carried out in the manner as described with **22**. The crude product obtained was purified by preparative TLC and recrystallized from acetone to give **29**, mp 158—159°.

Epimeric 2- and 4-Deuterio-5α-dihydrotestosterone *tert*-Butyldimethylsilyl Ethers (30, 31, 32, 33)——To each solution of **26**, **27**, **28** and **29** (50 mg) in pyridine (1 ml) was added CrO_3-pyridine complex (1: 10 w/v) (1 ml) and allowed to stand at room temperature for 5 hr. The reaction mixture was diluted with ether, washed with 10% AcOH, 5% $NaHCO_3$ and H_2O successively, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using hexane–ethyl acetate (15: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **30**, **31**, **32** and **33** (*ca.* 40 mg) as colorless needles, mp 133—134°.

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